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AURICULAR ENDOCARDITIS OF RHEUMATIC ORIGIN *

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In recent publications, attention has been directed by MacCallum[1] to certain changes occurring at times in the wall of the left auricle in rheumatic disease of the heart. The appearance of the lesion as described by him is very characteristic. Upon the wall of the auricle above the root of the posterior leaflet of the mitral valve, the endocardium is thickened, forming a corrugated patch, often with a rough, dull, fibrinous layer on the surface; at other times the surface seems not to be roughened by any thrombotic mass. In some instances the thickened patches appear to be very dense and scar-like as though representing a late or healing stage of an earlier process. Upon section, the great thickening of the lining wall of the auricle inside the muscular layer is readily seen. Histologically, the changes are equally characteristic. In the tissue about the auriculoventricular junction, and in the wall of the auricle, as well as in the roots of the valve, there is great edema and marked infiltration with wandering cells of many sorts. Most of these are mononuclears, but there are also many polymorphonuclear leucocytes, as well as numerous large Aschoff bodies composed of the typical cells. Because of the arrangement of the elastic lamellae, the Aschoff cells are forced into rows, giving the bodies a banded appearance. The Aschoff bodies are numerous throughout the whole extent of the patch of thickening, even extending into the sinus tissue and into the roots of the valve. The innermost layer of the auricular wall frequently has a

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hyaline appearance over the involved area; upon its surface is a dense film of fibrin. Outside the elastic layer the tissue reaching to the myocardium is densely infiltrated with wandering cells of various types, including in some cases a number of eosinophiles.

In 1898, Claude and Levaditi [2] demonstrated a case of chronic mitral endocarditis with what are described as "ulcerations" on the wall of the left auricle, the ulcerations being most abundant near the mitral orifice and reaching to the openings of the pulmonary veins. There were no thrombi on them; the surface was yellowish or gray in color, and calcium was deposited in the superficial portions. Histologically, capillaries were found to extend almost to the base of the ulcerations; and many round cells surrounded the capillaries. At other points the round cells were present together with elongate or stellate connective tissue cells. "Granular cells of Ehrlich" were seen also. Some of the smaller arteries showed marked endarteritis with almost complete obliteration of the lumen, and at the periphery of some of the vessels was a proliferation of granulation tissue. In the myocardium, granulation tissue was most marked about the large capillaries. The pericardium contained numerous vessels presenting the same inflammatory alterations as in the endocardium and myocardium. Bacteria could not be demonstrated. It is unfortunate that a history is not included with the report. In view of the fact that bacteria could not be demonstrated, that the mitral valve showed only chronic changes without a recent endocarditis and that there were no thrombi on the "ulcerations," it seems very probable that this case is one of rheumatic endocarditis of the auricle with calcification. Furthermore, the nature of the lesions, though incompletely described, in the myocardium and pericardium would tend to support this belief.

Harper [3] in an address before the Southern Medical Association at Lexington, Kentucky (1912), described the heart of a boy, 8 years of age, which he had studied in the Great Ormond Street Hospital for Sick Children, London. The child had had chorea. There were verrucous vegetations on the mitral and aortic valves, and the "endocardium of the left auricle was found to be thickened and wavy. This was considered evidence of a former endocarditis." (Case VII.) The report does not include a histologic examination. This was very probably a case of rheumatic endocarditis of the auricle, but the evidence presented is too scant to allow one to be certain.

In 1920 Hertel [4] in a discussion of parietal endocarditis gives a detailed description of a case of verrucose endocarditis with involvement of the left auricular wall (Case VI). Her description presents clearly the gross picture and histologic changes. Upon the mitral valve were vegetations, in part wart-like and in part more polypoid. These vegetations extended to the base of the valve, and from there were continued upon the auricle. The surface of the auricle in places was smooth; in other places there were parallel, transverse or irregularly running ridges and upon and between them, warty masses. The verrucae and ridges extended high up into the auricle. The foramen ovale could scarcely be recognized.

Histologically, the verrucae were made up of fibrin. In the deeper part of the auricular endocardium were bands of connective tissue, staining red with Van Gieson's picro-fuchsin stain, while near the surface the connective tissue stained less intensely with the fuchsin, even appearing in places yellow. Small masses of cells were present, made up of connective tissue cells, polymorphonuclear leucocytes, lymphocytes and plasma cells; such cells being found in the superficial as well as the deeper parts of the endocardium. Many capillaries invaded the endocardium from the myocardium in a perpendicular fashion, and were surrounded by mantles of these cells. Bacteria could not be found. The continuity between the lesions of the mitral valve and those of the auricular wall is emphasized in the discussion of the case. The clinical history is not included in the report, but the case is undoubtedly one of rheumatic disease with involvement of the wall of the left auricle. The lesion as described would seem to coincide with the later stages in rheumatic auricular endocarditis.

Swift [5] states that "in the mural endocardium lesions are found without a deposit of fibrin on the surface, but showing a type of infiltration closely resembling that of Aschoff bodies." No mention is made of the situation of these lesions, nor of their frequence in the auricular and ventricular endocardium.

Since the publication by MacCallum, Stewart and Branch [6] have described a case of rheumatic endocarditis of the left auricle, with calcification and Aschoff bodies.

In the past five years there have been thirty-one cases of rheumatic valvular endocarditis demonstrated at necropsy in this hospital, and of these, nine have shown involvement of the auricular endocardium.

Gross Pathology of Rheumatic Auricular Endocarditis

The gross appearance of the auricular rheumatic endocarditis is as distinctive as that of the verrucose vegetations on the valves, or the myocardial and pericardial changes.

In the simplest form the lesion is confined to an area just above the non-aortic or posterior leaflet of the mitral valve. Here the endocardium is made irregular by low ridges or hillocks often closely crowded together to form a plaque 3 cm. or more in extent. At other times these elevations are more widely separated from each other. When closely aggregated, the surface has a furrowed appearance and the furrows, while having no definite pattern, frequently run roughly perpendicular to the ring of the valve (Figs. 1 and 2). Similar, but somewhat less distinct, ridges can usually be traced downward across the auricular surface of the valve leaflet, to merge with the vegetations near the margin of the cusp (Fig. 1).

The surface of these auricular elevations may be quite smooth and glistening, or slightly dull and irregular; it is occasionally roughened by tiny, dull, yellowish flecks or a little blood clings to it. Rarely, more distinct projections resembling vegetations are seen. The color is variable also. In the more acute lesions these thickenings are tawny gray; in the older stages, grayer and translucent.

When the process is more extensive, flat or delicately ridged plaques of yellow color are found in many parts of the auricle. They often surround the orifices of the pulmonary veins but do not extend into them. Seldom is the surface of the fossa ovalis involved. In one instance almost the entire endocardial surface of the auricle was lifted up to form a single, flat plaque with a faintly wrinkled surface (Fig. 3). This condition apparently resulted from the coalescence of many smaller areas. Not infrequently the elevations are to be seen in the first portion of the auricular appendage. The edges of these elevated areas may be quite abrupt or they may fall away in an almost imperceptible fashion.

When a section is made through the areas of thickening, certain changes can be readily discerned. The endocardial layer is found to be considerably increased in thickness; it is gray or yellowish gray, the yellow color being most pronounced in the more acute lesions. In some instances, only the very superficial portion seems to be the site of the changes, for the gray, translucent, normal-appearing

endocardium is easily traced as a regular layer beneath the superficially placed yellowish mass. Yet, from the histologic study of such cases, it is found that this normal-appearing portion of the endocardium beneath the plaque is often much altered. In other instances the endocardium is involved throughout its entire width, for it no longer appears as an even gray band but is irregular and distorted. Little irregularity can be made out, however, in the outline of the lower or myocardial border.

In the later stages these hillocks and ridges flatten down and frequently become less distinct (Figs. 4 and 5). They are readily recognized as pale gray thickenings, sometimes containing thin plates of calcium. Section through these raised areas shows that the endocardium is widened, dense, homogeneous and gray, with the deposits of calcium in the superficial part.

Histology of the Normal Endocardium

The endocardium of the left auricle has been described by Königer [7] and separated by him into the following layers: (1) endothelium, (2) a delicate subendothelial layer of connective tissue, (3) a thick layer of elastic fibers, with connective tissue, and (4) a connective tissue layer with abundant elastic fibers.

Nagayo [8] further extended the differentiation of the auricular endocardial structure, and divided the endocardium as follows: (1) endothelium, (2) inner connective tissue layer, (3) middle layer of elastic and connective tissues, and (4) outer connective tissue layer, with a few coarse elastic fibers. Between the last two layers he demonstrated the presence of smooth muscle, either as scattered fibers or more or less collected together into bundles. He aptly likened the structure of the endocardium to that of the entire wall of the larger arteries.

It has seemed to us that the innermost elastic fibers are often collected into a fairly definite bundle resembling the internal elastic lamella of the larger arteries.

The structure of the endocardium of the right auricle is similar to that of the left, though in the former situation the endocardium is nowhere as thick as in the latter.

Histology of Rheumatic Auricular Endocarditis

At the site of the thickened areas, great changes are to be made out in the endocardium. In many of the more acute lesions, large accumulations of small mononuclear cells, many polymorphonuclear leucocytes and occasionally a few eosinophiles are found in the outer half of the endocardium. At times these infiltrations reach to the inner surface.

More striking than these are collections of large cells generally forming a palisade along a band of hyaline material, their nuclei being perpendicular to the band (Figs. 6, 7 and 8). Frequently this band of hyaline substance coincides with the layer of connective tissue just beneath the endothelium. In some instances this subendothelial connective tissue is swollen and hyaline over a considerable area without any cells being accumulated about it. At other times similar though shorter hyaline bands lie in the elastic-connective tissue layer. Occasionally one of these bands will bend abruptly, almost at right angles, yet these larger cells follow its turnings and preserve their perpendicular position with regard to it.

In addition to the striking palisade arrangement of these cells, smaller groups of them are found which have a radial arrangement about a central mass of the hyaline material, probably the result of a transverse instead of a longitudinal section of the band about which they are accumulated. Somewhat similar, though less regularly arranged, accumulations of these large cellular elements are collected about foci consisting of fragments of swollen collagen.

The cytoplasm of these prominent cells stains faintly blue in the hematoxylin-eosin preparations. The nuclei are vesicular and oval, or occasionally slightly lobed, and some contain a large stellate nucleolus; in others the chromatin is in smaller particles. The nuclear membrane is wide and stains deeply. In preparations stained with methyl green-pyronin, many of these cells stand out sharply because of the brilliant red cytoplasm and pale green nucleus. They resemble in morphology and staining reaction the large cells taking part in the formation of the Aschoff bodies in the myocardium and other places. These lesions probably are modified Aschoff bodies, their shape being determined by the laminated structure of the tissue in which they occur. In sections treated with Weigert's elastic tissue and hematoxylin-Van Gieson's picro-fuchsin stains, the

material about which these cells are accumulated stains intensely with fuchsin and is obviously swollen, degenerated collagen. Accompanying this degenerated collagen are occasionally well preserved or slightly swollen elastic fibers, the cells then surrounding both.

Other sections at times show only cellular accumulations of another sort. These are made up of cells with indistinct outline and having compressed, distorted, curved and elongate nuclei. With them are many polymorphonuclear leucocytes, a few plasma and small round cells, occasional eosinophiles and also pale cells with vesicular nuclei containing a few small particles of chromatin (Figs. 9 and 10). The number of polymorphonuclear leucocytes is quite variable; in some instances they are so numerous that they are the predominant cells, tending to obscure the other cellular elements.

These cell aggregations may be found in any portion of the endocardium, but are perhaps more often present in the inner half, or even between the main elastic-connective tissue layer and the endothelium. The distorted cells are most probably the result of degeneration of the large pale cells with vesicular nuclei, which are apparently large mononuclear wandering cells. They do not correspond morphologically with the Aschoff cells, neither do they stain with methyl green-pyronin as do the Aschoff cells. Furthermore, as a rule, no Aschoff cells can be demonstrated with the methyl green-pyronin stain in these cell masses.

The arrangement of the large distorted cells in such accumulations is characteristic. Their nuclei are usually directed perpendicularly to the endocardial surface. Cell degeneration takes place readily, for nuclear fragments lie scattered about. These cell aggregations are often the cause of the ridges and the irregularities of the auricular surface.

The endothelium over the sites of these changes is frequently intact. At other times there is a thin layer of fibrin upon the surface where the endothelial cells have disappeared, and now and then this fibrin is collected into verruca-like vegetations (Fig. 11).

The elastic fibers are widely separated and pushed apart by the various cell accumulations. Often the fibers are slightly swollen and fragmented, or they may pass intact through the cell mass, though somewhat bent out of their usual course. The internal elastic lamella can be readily made out either passing through a mass of the infiltrating cells or curving upward over them and preserving

its normal relationship to the endothelium (Fig. 12). Sometimes the fibers of this lamella are spread apart by the cells, the innermost fibers passing over the accumulated cells, the remainder through the cell mass, uniting on either side of it. Now and again the innermost fibers are ruptured. Delicate elastic fibrils, possibly newly formed, can be discerned between the cells.

In the acute stages there is considerable edema, and fibrin is found in varying amounts among the distorted cells, but none can be demonstrated in the homogeneous material about which the Aschoff cells are gathered. Fibrin is readily demonstrated in places upon the surface of the endocardium over those areas where the endothelium has disappeared, and is also present in considerable quantity in the edematous vascular zone near the myocardium.

Bacteria have not been demonstrated in either Gram or Levaditi preparations.

Healing and repair take place readily. From the myocardium, capillaries penetrate into the outer half of the endocardium but they have not been found to pass beyond the mid-portion of it. Surrounding these new vessels are many small mononuclear cells, plasma cells and a few polymorphonuclear leucocytes. In the superficial part, especially between the main elastic-connective tissue layer and the endothelium there appear cells resembling fibroblasts with their nuclei directed toward the surface (Fig. 13). These cells form an avascular tissue, frequently staining yellow with the Van Gieson picro-fuchsin stain. The fibrin upon the surface of the endocardium is invaded and surrounded by such cells, hyaline masses of it being seen in the superficial part of this new tissue. In this compact new tissue, delicate elastic fibrils appear, often turning upward like a fringe into the deepest portion (Fig. 14). The internal elastic lamella may pass over such a scar, giving the impression that the scar marks the site of one of the accumulations of distorted cells and polymorphonuclear leucocytes; usually it passes beneath the plaque. Calcium is frequently deposited in the superficial portion of the endocardium where the scarring is taking place, and it may be in thin plates or small nodular clumps.

The elastic fibers in the mid-portion of the endocardium are stretched and taut beneath the new tissue, and recall very strikingly the appearance of the elastic fibers beneath a sclerotic plaque in the aorta.

As the healing progresses, the characteristic cells disappear and finally there is left the dense avascular scar in the superficial part, sometimes containing calcium. Also occasional collections of small wandering cells are found at the junction of the endocardium and myocardium; such cell aggregations appear to persist for a long time. The end result gives little indication of the striking cell infiltration and accumulations which have gone before.

It is not to be supposed that the changes enumerated above take place in definite stages and are carried through to completion before other acute lesions appear. Acute, healing and healed lesions are frequently encountered in the same section indicating that there have been repeated insults. Neither does one usually find the various cell types occurring to the exclusion of the others. MacCallum has emphasized the presence of the Aschoff cells and bodies as being the most characteristic feature, and they do form a conspicuous part of the lesion. In the case reported by Stewart and Branch,[6] only Aschoff bodies and calcium deposits were found. From our studies it has seemed to us that the masses of distorted cells and polymorphonuclear leucocytes constitute a feature as prominent and distinctive as the Aschoff bodies. It is probable that many of the plaques of calcium found in the auricular endocardium in chronic mitral disease mark the sites of previous acute rheumatic lesions.

Rheumatic Endocarditis of the Right Auricle

Only one instance of rheumatic endocarditis of the right auricle has come to our attention. In this case with extensive endocardial changes in the left auricle, a chain of tiny verrucae was present along the anterior margin of the fossa ovalis.

From the foregoing study, one is convinced that there occur in the endocardium of the auricle, lesions which are distinctive, both grossly and histologically, of rheumatic endocarditis. The endocardium of the left auricle is more frequently the site of the lesions than that of the right auricle. In the series of cases studied, the left auricle was involved in approximately one-third of the cases of rheumatic valvular endocarditis.

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Résumé of Cases

Case I. Acute Rheumatic Endocarditis of Left Auricle.

Autopsy 9168, history 51744. L. D., male, age 14. One attack of rheumatism, duration two weeks, seven years before death. Scarlatina four years ago. Dyspnea on exertion for two years.

Heart: Greatly enlarged. Pericardium everywhere adherent to heart. Verrucose vegetations on tricuspid, mitral and aortic valves. Mitral valve scarred. Endocardium of left auricle thickened to form a large plaque whose surface is somewhat wrinkled and irregular. The elevated area is yellow in color. The thickening involves almost the entire auricle, extending into the auricular appendage and downward upon the surface of the mitral valve (Fig. 3). The process stops at a little distance from the orifices of the pulmonary veins. Myocardium scarred.

Histologic Examination: In the endocardium of the left auricle are bands of hyaline material bordered by Aschoff cells. There are also Aschoff cells about collagen fragments. Large areas densely infiltrated with small mononuclear cells, polymorphonuclear leucocytes and a few eosinophiles; capillaries are penetrating from myocardium into these cell accumulations. No vegetations on the surface. Elastic fibers are pushed apart by the infiltrating cells, and are distorted and fragmented. Delicate elastic fibrils in the areas of cell infiltration. Aschoff bodies in the myocardium.

Case II. Healed Rheumatic Endocarditis of Left Auricle.

Autopsy 9179, history 45652. L. W., male, age 6. Acute rheumatic fever at 4 and 5 years of age. Mitral insufficiency.

Heart: Enlarged. Fibrinous pericarditis. Tricuspid valve thickened. Endocardium above posterior leaflet of mitral valve is thrown up into low gray ridges, with smooth and shining surface. Mitral and aortic valves scarred.

Histologic Examination: Focal accumulations of small round cells at junction of endocardium and myocardium. In mid-portion of endocardium small groups of cells, with indefinite outline, large vesicular nuclei and stellate nucleoli. Internal to the elastic-connective tissue layer is a mass of loose avascular connective tissue, within which are a few delicate elastic fibers. Aschoff bodies in myocardium. Organizing fibrinous pericarditis.

Case III. Healed Rheumatic Auricular Endocarditis.

Autopsy 9370, history 32823. J. V., male, age 6. Measles and chickenpox. Tonsillitis repeatedly. Attacks of rheumatism three, two and one years before death.

Heart: Enlarged. No pericarditis. Verrucae on mitral and aortic valves. Above posterior leaflet of mitral valve the endocardium of the auricle is thickened to form rounded low hillocks with gradually sloping margins and smooth surface (Fig. 4). No scars visible in the myocardium.

Histologic Examination: Between the internal elastica and the endothelium are accumulations of avascular fibrous tissue, with nuclei directed perpendicularly to the endothelial surface. Elastic fibers do not appear irregular. Aschoff bodies in myocardium.

CASE IV. ACUTE RHEUMATIC ENDOCARDITIS OF LEFT AURICLE.

Autopsy 9408, history 56872. M. F., female, age 3. Acute rheumatic fever, knees and ankles involved, one year before death. Scarlatina shortly after the rheumatic fever. Dyspnea on exertion for four months.

Heart: Hypertrophied. Pericardium everywhere adherent to heart. Verrucae on tricuspid, mitral and aortic valves. Endocardium of left auricle above posterior leaflet of mitral valve is thickened over an area of 3 cm., the plaque reaching into first part of appendage and across valve leaflet to line of closure (Fig. 1). Surface of this thickened area traversed by many shallow depressions. Plaque is pale yellow; surface is only slightly less smooth than that of the endocardium in uninvolved portion of the auricle. Many smaller tawny yellow plaques in other parts of the auricle, near fossa ovalis and orifices of pulmonary veins. Sections through these plaques show the deeper portion of the endocardium to be slightly opaque, while the bulging plaque is edematous and yellowish white in color. Myocardium scarred.

Histologic Examination: The changes are quite identical with those described in Case I. Numerous Aschoff bodies and large scars in the myocardium.

CASE V. HEALED AND ACUTE RHEUMATIC ENDOCARDITIS OF LEFT AURICLE.

Autopsy 9471, history 58720. W. G., male, age 27. Acute rheumatic fever at age of 21, with cardiac involvement. Palpitation for two months before first admission to hospital. One year later dyspnea and orthopnea.

Heart: Greatly enlarged, weighing 1080 gm. Pericardium everywhere adherent to heart. Mitral valve thickened. Verrucae on mitral and aortic valves. Above the posterior leaflet of the mitral valve, over an area 4 × 2 cm., are many elevated yellowish plaques and ridges. Similar though smaller yellow ridges extend downward from this large aggregation across the auricular surface of the posterior leaflet of the valve (Fig. 2). The margins of the plaques are quite abrupt at the upper border. Surface of the plaques is slightly dull in a few places. Section shows that the thin gray endocardial layer widens out at the sites of these plaques, yet it can be traced as a gray band beneath the superficial yellow portion. Along the anterior margin of the fossa ovalis in the right auricle is a row of tiny verrucae.

Histologic Examination: Characteristic rheumatic valvular endocarditis. In some places on the surface of the auricle are small hyaline masses which are being surrounded by a loose connective tissue. In other places between the elastic-connective tissue layer and the endothelium are masses of connective tissue cells with their nuclei directed perpendicularly to the surface (Fig. 13). Delicate elastic fibrils pass up into this connective tissue. No Aschoff bodies found in several sections of the myocardium.

CASE VI. ACUTE RHEUMATIC ENDOCARDITIS OF LEFT AURICLE.

Autopsy 9529, history 60354. A. S., male, age 14. No history of rheumatism. Frequent attacks of sore throat. Precordial pain, dyspnea and fever one year ago.

Heart: Pericardial sac everywhere bound to the heart by fibrous adhesions. Heart enlarged. Verrucae on the tricuspid, mitral and aortic valves. Delicate elevated yellowish plaque extends across the auricular surface of the posterior leaflet of the mitral valve and merges with similar larger plaque on the endocardial surface of the auricle. This latter plaque appears to be formed by the

coalescence of many smaller ones. The surface is distinctly roughened and dull, and is flecked with red. The plaque reaches to the openings of the pulmonary veins. Isolated oval or rounded elevated areas, 3 to 4 mm. in diameter, occur in other parts of the auricle. One of these at the orifice of the auricular appendage is somewhat larger and is continued downward across the auricular surface and upon the anterior leaflet of the mitral valve. On section the main portion of the endocardium passes in a regular fashion beneath the gray translucent thickenings in the superficial part.

Histologic Examination: The lesion is intense. Aschoff cells are collected about bands of hyaline material (Fig. 6). In other areas there is intense infiltration with polymorphonuclear leucocytes, mononuclear cells, a few eosinophiles and aggregations of cells with distorted compressed elongate nuclei (Figs. 9 and 10). The nuclei of these latter cells are directed perpendicularly to the surface. The endothelium is intact over some of these cell accumulations; in other places masses of fibrin are on the surface (Fig. 11). In yet other places are masses of avascular connective tissue between the endothelium and main elastic-connective tissue layer, with many delicate elastic tissue fibrils in the deeper part of the connective tissue mass (Fig. 14). The elastic fibers are spread apart and fragmented where these various cells are accumulated (Fig. 12). Delicate elastic fibrils are found in the cell masses. The internal elastica is lifted up by groups of the distorted cells. Many capillaries arising in the myocardium are invading the outer portion of the endocardium and are surrounded by small round cells, plasma cells and a few polymorphonuclear leucocytes and eosinophiles. The myocardium just beneath the endocardium is edematous. Many Aschoff bodies are found in the myocardium.

CASE VII. HEALED RHEUMATIC ENDOCARDITIS OF THE LEFT AURICLE.

Autopsy 9534, history 43778. K. F., female, age 37. Rheumatic fever at age of 13. Dyspnea and palpitation began four years before death.

Heart: Moderately enlarged, weighing 450 gm. Mitral valve thickened and the edge rolled under. Over the non-aortic leaflet of the valve the auricular endocardium is thrown up into irregular ridges and folds, and these are continued for a short distance across the auricular surface of the valve (Fig. 5). These thickenings are translucent and faintly yellow.

Histologic Examination: The elevations are composed of avascular connective tissue lying internal to the main elastic-connective tissue layer of the endocardium. The internal elastic lamella lies beneath these areas and is somewhat interrupted as it passes below them. Many delicate elastic fibrils are present in the connective tissue masses. Aschoff bodies occur in the myocardium.

CASE VIII. HEALED RHEUMATIC ENDOCARDITIS OF THE LEFT AURICLE WITH CALCIUM DEPOSITS.

Autopsy 9607, history 52993. J. F., male, age 17. Rheumatic fever four years before death. Dyspnea, edema of legs and palpitation for three months.

Heart: Enlarged, weight 710 gm. Tricuspid and mitral valves are thickened and have verrucae upon them. Above the posterior leaflet of the mitral valve, the endocardium of the left auricle over a large area is thrown up into irregular grayish yellow folds and rounded plaques; calcium is deposited in the superficial portion of these folds.

Histologic Examination: Internal to the main elastic-connective tissue layer, the endocardium is irregularly thickened by masses of dense connective tissue

in which many delicate elastic fibrils are found, usually directed at right angles to the surface. Here and there are coarse elastic fibers just beneath the endothelium and parallel to it; these are apparently remnants of portions of the internal elastic lamella. Calcium is deposited in the masses of dense connective tissue. Localized collections of lymphocytes are present in the endocardium near the myocardial surface.

CASE IX. ACUTE RHEUMATIC ENDOCARDITIS OF LEFT AURICLE.

Autopsy 9627, history 48682. S. G., male, age 9. Two attacks of rheumatic fever at 5 and 7 years of age. Mitral and aortic insufficiency; mitral stenosis.

Heart: Greatly enlarged. Pericardial surface covered with a thick fibrinous exudate. Tricuspid and pulmonic valves normal. Left auricle is enlarged. Numerous slightly elevated yellow areas in the endocardium in many parts of the auricle, but especially marked above the posterior leaflet of the mitral valve. These yellow areas are glistening, though frequently the surface is a little wrinkled. Marked thickening and distortion of the mitral leaflets with festoons of verrucose vegetations near the free border. Aortic leaflets thickened; chains of verrucae upon them.

Histologic Examination: At the site of the plaques in the left auricle the endocardium is greatly thickened and infiltrated with cells consisting of large mononuclears, small round cells and many polymorphonuclear leucocytes. Close to the surface is a band of swollen and fragmented collagen. In many parts there are no cells about this band. In other places hyaline bands are accompanied by palisades of Aschoff cells (Figs. 7 and 8). In yet other places are dense accumulations of cells; some of the cells are large with pale vesicular nuclei, others have distorted compressed nuclei. With these are many polymorphonuclear leucocytes and a few eosinophiles. In the deeper part of the endocardium are numerous capillaries about which are large mononuclear cells, lymphocytes and a moderate number of polymorphonuclear leucocytes. The endothelium is intact on the surface. In the myocardium are great numbers of characteristic Aschoff bodies. The epicardium is covered with a thick layer of fibrin undergoing organization.

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DESCRIPTION OF PLATES

PLATES 1–9

(For more detailed description of gross specimens, see résumé of cases.)

FIG. 1. Autopsy 9408. Acute rheumatic endocarditis of mitral valve and left auricle. Adherent pericardium.

FIG. 2. Autopsy 9471. Acute rheumatic endocarditis of mitral valve. Acute and healed rheumatic endocarditis of left auricle. Adherent pericardium.

FIG. 3. Autopsy 9168. Rheumatic endocarditis of mitral valve. Extensive acute rheumatic endocarditis of left auricle. Adherent pericardium.

FIG. 4. Autopsy 9370. Rheumatic endocarditis of mitral valve. Healed rheumatic endocarditis of left auricle.

FIG. 5. Autopsy 9534. Healed rheumatic endocarditis, with great scarring and thickening of mitral valve. Healed rheumatic endocarditis of left auricle.

FIG. 6. Autopsy 9529. Acute rheumatic endocarditis of left auricle. Hyaline bands surrounded by Aschoff cells. (Hematoxylin-eosin stain.)

FIG. 7. Autopsy 9627. Acute rheumatic endocarditis of left auricle. Aschoff cells along bands of hyaline material in mid-portion of endocardium. Accumulation of wandering cells in deeper part of endocardium. (Hematoxylin-eosin stain.)

FIG. 8. High power of outlined field in Fig. 7, showing character and arrangement of cells around hyaline material. (Hematoxylin-eosin stain.)

FIG. 9. Autopsy 9529. Acute rheumatic endocarditis of left auricle. Dense infiltration of endocardium with wandering cells of many varieties. (Hematoxylin-eosin stain.)

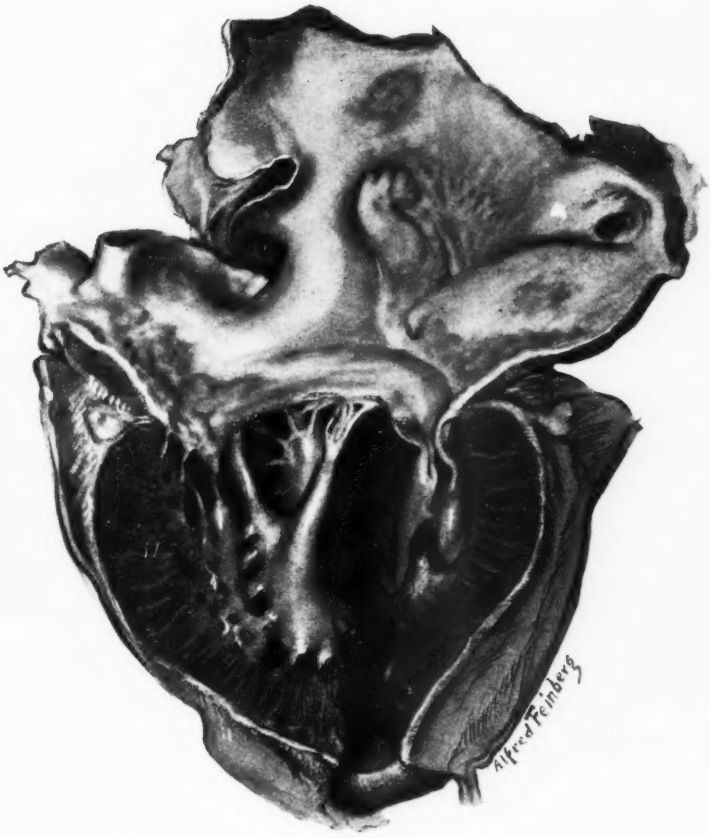
FIG. 10. High power of outlined field in Fig. 9, showing large mononuclear wandering cells, polymorphonuclear leucocytes, small round cells and cells with distorted, compressed, elongate nuclei. (Hematoxylin-eosin stain.)

FIG. 11. Autopsy 9529. Acute rheumatic endocarditis of left auricle. Vegetation composed of fibrin strands. (Hematoxylin-eosin stain.)

FIG. 12. Autopsy 9529. Acute rheumatic endocarditis of left auricle. Separation and fragmentation of elastic fibers following cellular infiltration of endocardium. (Weigert's elastic tissue-hematoxylin-Van Gieson stain.)

FIG. 13. Autopsy 9471. Healed rheumatic endocarditis of left auricle. Mass of avascular connective tissue internal to elastic-connective tissue layer of endocardium. (Weigert's elastic tissue-hematoxylin-Van Gieson stain.)

FIG. 14. Autopsy 9529. Healed rheumatic endocarditis of left auricle. Avascular connective tissue internal to elastic-connective tissue layer of endocardium. Fringe of delicate elastic fibrils in deepest part of scar. Elastic fibers underlying new tissue stretched and taut. (Weigert's elastic tissue-hematoxylin-Van Gieson stain.)



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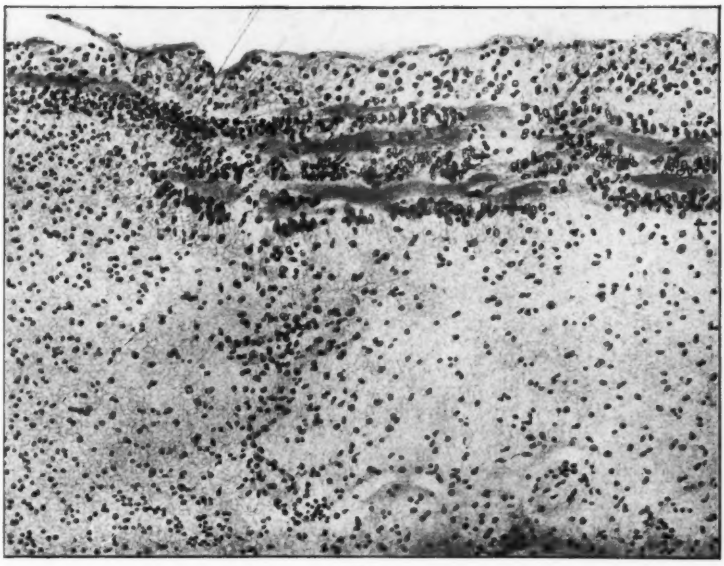

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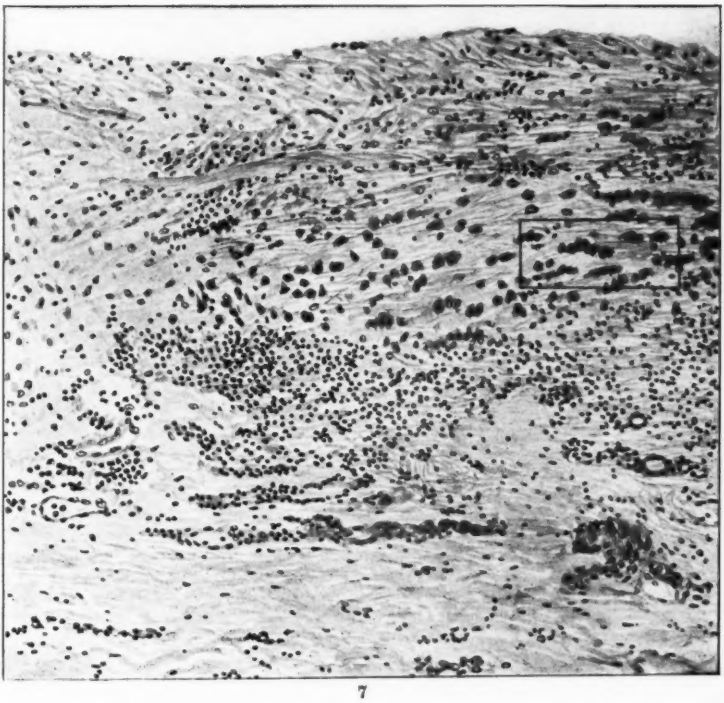

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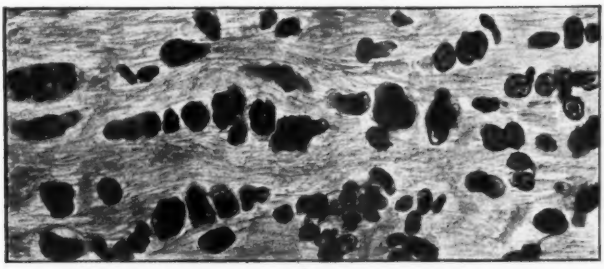

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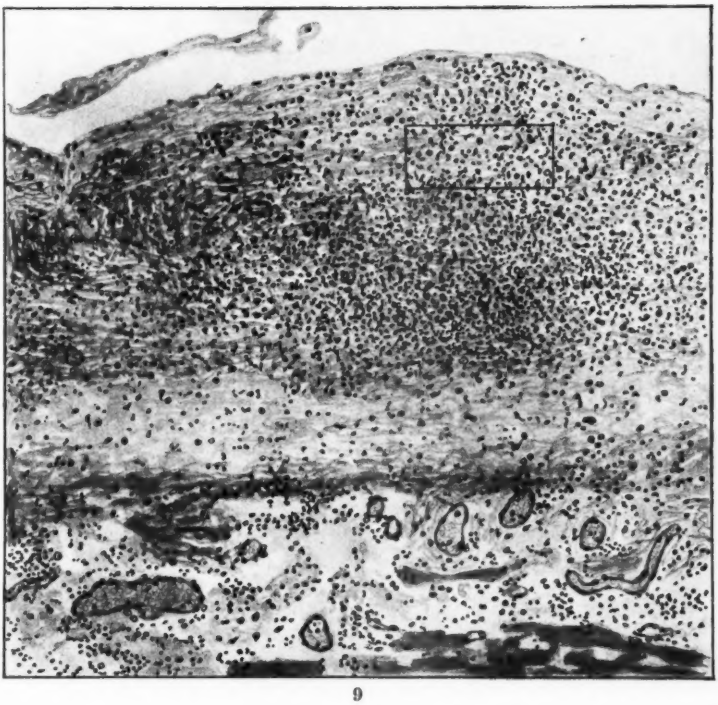

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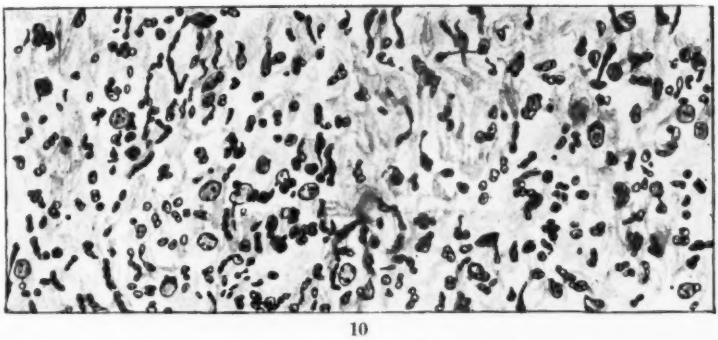

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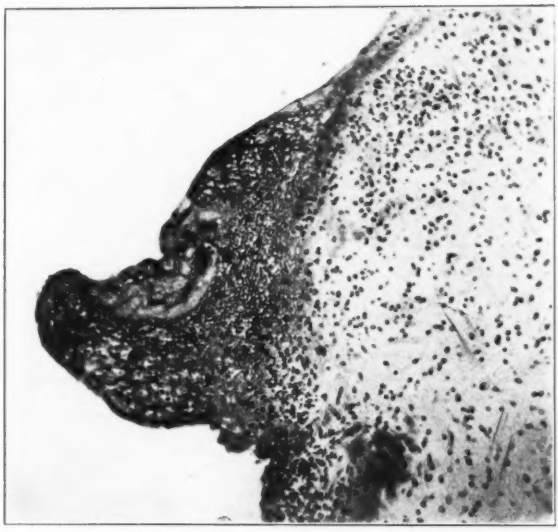
VonGlahn Auricular Endocarditis of Rheumatic Origin



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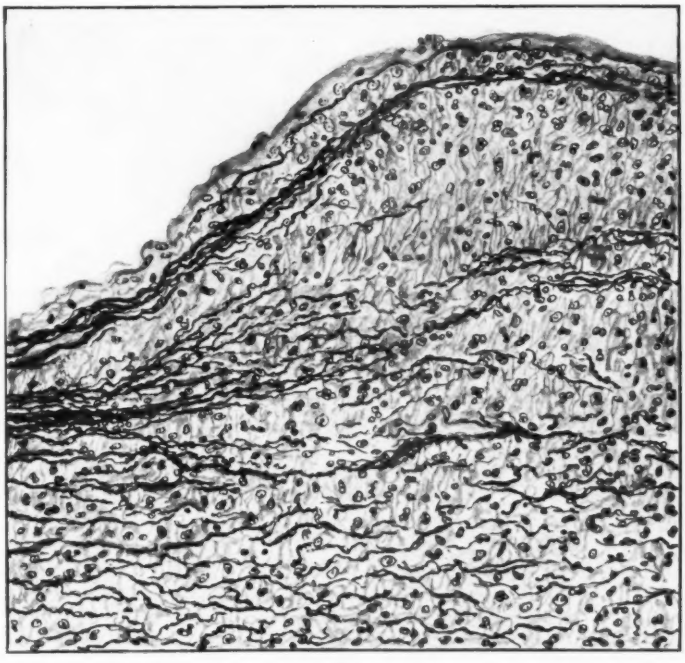

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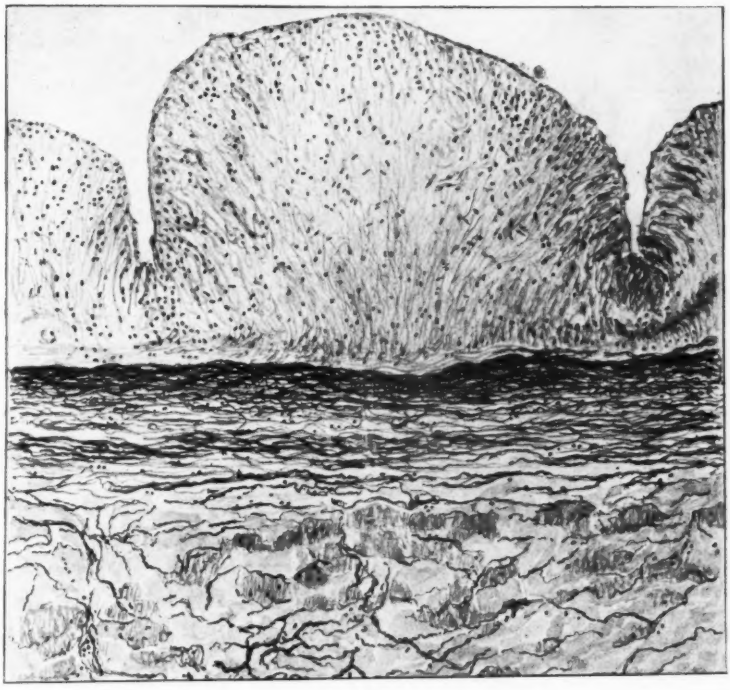

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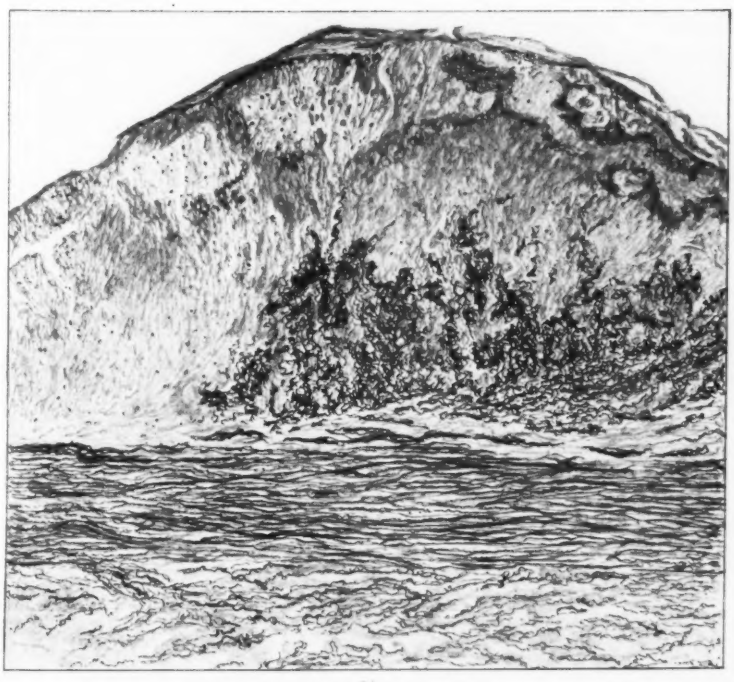

14

VonGlahn Auricular Endocarditis of Rheumatic Origin

A CASE OF RHEUMATIC AORTITIS WITH EARLY LESIONS IN THE MEDIA*

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and

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In a previous paper (1924) [1] dealing with the lesions of the aorta in rheumatic infections, two types of pathologic change were described as characteristic: the occurrence of Aschoff bodies or isolated Aschoff cells in the adventitia; and the presence of flame-shaped scars about the nutrient vessels of the media. In most of the cases examined, these medial cicatrices were dense and acellular, and were interpreted as healed or healing lesions. The material then available thus offered no opportunity to decipher the earlier changes leading up to the formation of the scars.

A case recently studied, in which the aortic lesions were acute and of unusual intensity, illustrates an earlier stage in the development of the medial alterations and is reported for that reason.

S. C., age 9, white, male (history 48682, autopsy 9627), was first admitted to the Presbyterian Hospital on Jan. 10, 1921, with painful joints in the arms and legs following exposure to cold three weeks previously. The course thereafter until his death on April 17, 1925, was typical of rheumatic fever with remissions, but with progressive involvement of the heart and pericardium. Blood cultures on his first admission were sterile.

The anatomic findings may be summarized as follows: *rheumatic endocarditis (mitral and aortic valves, and left auricle); rheumatic myocarditis; rheumatic pericarditis, organizing; serofibrinous pleurisy; cardiac hypertrophy and dilatation; chronic passive congestion of viscera; asciles.*

Only the aorta need be described minutely; grossly it shows nothing abnormal. Microscopically, however, striking changes are found, both in the adventitia and in the outer two-thirds of the media. The endothelial cells of the vasa vasorum are swollen. About these vessels are collections of small round cells and plasma cells together with many polymorphonuclear leucocytes. The lumen of some of the smaller vessels is filled with polymorphonuclears. In many parts

* Received for publication November 12, 1925.

of the adventitia, the cellular infiltration is diffuse and intense, with the polymorphonuclear leucocyte as the predominant cell.

In areas where the infiltration of wandering cells is less profuse, the connective tissue has taken on a peculiar appearance: the collagen bundles are swollen, and between them the connective tissue cells have somewhat the character of fibroblasts with basic staining cytoplasm and swollen nucleus. Multinucleated cells are not uncommon.

In none of the numerous blocks studied are there found definite Aschoff bodies.

The medial changes are essentially of the same character as those described in the adventitia, save that their distribution appears to be determined by the course of the nutrient vessels. The nutrient arterioles are thick walled, due in part to the swelling, and in some instances to the heaping up of the endothelium; in part also to the cellular infiltration and perhaps edema of their walls. About the nutrient vessels, pushing apart the adjacent elastic fibers, are profuse collections of cells, amongst which one can distinguish small lymphoid elements, polymorphonuclear leucocytes (often in considerable numbers) and larger elements with vesicular nuclei, prominent stellate nucleolus and strongly basophilic cytoplasm (Fig. 1). With the methyl green-pyronin stain, these larger cells react as do the Aschoff cells. Often these are aligned in rows between the elastic fibers (Fig. 2). A few multinucleated cells are seen.

The collagen fibers within and adjacent to the cellular infiltrations are swollen and more intensely stained with eosin or fuchsin than the normal collagen of the aorta. The elastic lamellae in these early cellular lesions are displaced, but not destroyed.

No bacteria could be demonstrated. Histologic study of the other tissues in this case, it should be stated, give no indication of a bacterial infection. The character of the valvular and myocardial disease is in all respects typical of rheumatic infection, and there are no embolic lesions.

A study of this case supports the view previously expressed that the perivascular medial scars are the sequel of an earlier focal perivascular inflammatory lesion.

We are indebted to Dr. Walter W. Palmer for permission to transcribe the clinical record, and to Mr. Alfred Feinberg for the drawings.

REFERENCE

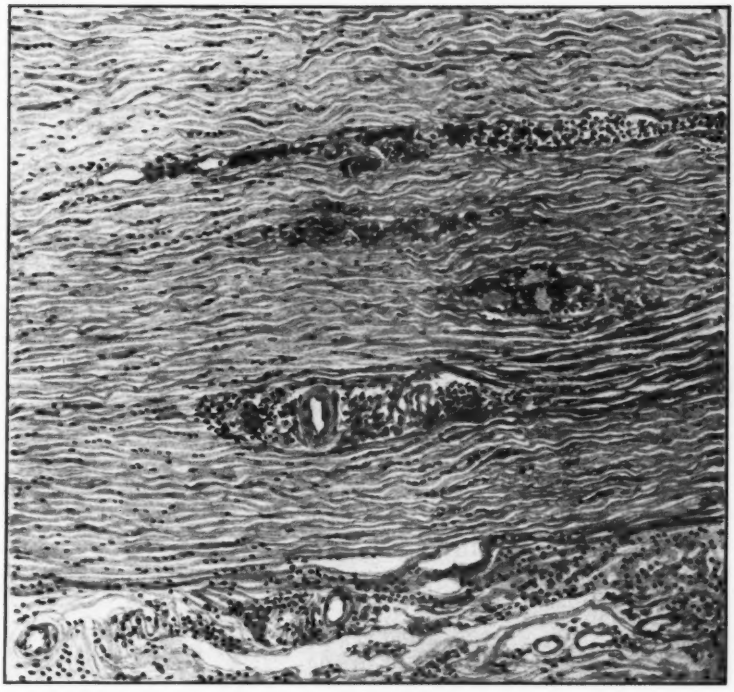
1. Pappenheimer, A. M., and VonGlahn, W. C. *J. Med. Res.*, 1924, xliv, 489–505.

DESCRIPTION OF PLATE

PLATE 10

FIG. 1. Acute rheumatic aortitis. Cellular infiltration about penetrating vessels in media.

FIG. 2. Acute rheumatic aortitis. Polymorphonuclear leucocytes and large cells with basophilic cytoplasm about penetrating vessels in media. Some of the large cells have multiple nuclei.



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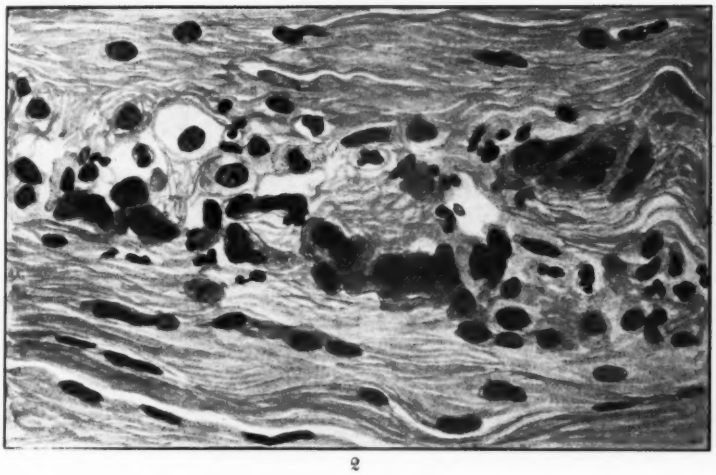

2

Pappenheimer and VonGlahn Rheumatic Aortitis

STUDIES ON COMPENSATORY HYPERTROPHY OF THE THYROID GLAND

VII. FURTHER INVESTIGATION OF THE INFLUENCE OF IODIN ON HYPERTROPHY OF THE THYROID GLAND WITH AN INTERPRETATION OF THE DIFFERENCES IN THE EFFECTS OF IODIN ON THE THYROID GLAND UNDER VARIOUS PATHOLOGIC CONDITIONS *

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In former investigations we have shown [1] in a relatively limited number of experiments that, contrary to the generally accepted view, administration of potassium iodid does not prevent compensatory hypertrophy in the thyroid gland of the guinea-pig. Considering the theoretical and practical importance of definite knowledge of the action of iodin on the thyroid under various conditions, we continued our investigations especially with a view of determining whether iodin may not only not prevent hypertrophy but also whether it may not even tend to increase it. We, therefore, carried out altogether seven series of experiments on 148 guinea-pigs, of which 72 received KI after previous removal of the greater part of the thyroid, and 76 served as controls. We also extended the time during which the iodin was allowed to act in some cases to approximately sixty days. Amounts of iodin varying in different experiments between 0.1 and 0.025 gm. KI were given each animal daily.

In order to summarize our results, it will be necessary to adopt a certain classification of the grades of hypertrophy. As we have done previously, we shall distinguish six types.[2] They are briefly as follows.

Grade I. Highest degree of hypertrophy. Entire liquefaction and absorption of colloid. Acinus cells high, cylindrical epithelium. Mitoses often present. Irregular acini; papillae present.

Grade II. Marked hypertrophy, similar to Grade I. Acini often form slits. In some peripheral acini, some pale soft colloid.

Grade III. Decided hypertrophy. Somewhat irregular acini; enlarged acinus cells, often granular, sometimes with mitoses. Colloid

* Received for publication Nov. 21, 1925.

diminished in quantity and consistency. Some acini form slits, but in the majority the colloid is merely diminished in quantity, softened, adherent and vacuolar. In a few acini the colloid may be more solid and retracted.

Grade III–IV. Acini slightly irregular; some with papillae, others regular in shape and lumen often wide. Acinus cells still moderately enlarged, sometimes with distinctly visible granules. There may be mitoses or amitoses. Usually colloid is distinctly softened, bluish, vacuolar, adherent or retracted; often somewhat diminished in quantity.

Grade IV. Very moderate hypertrophy. Usually regular, here and there some irregular acini. Walls of adjoining acini may be partially dissolved, resulting in the formation of spurs. Acinus cells somewhat enlarged, at least over wider areas. Mitoses rare. Colloid slightly softened, pale, bluish, usually vacuolar and attached to the epithelium. In some acini it may be more solid and retracted.

Grade IV–V. Trace of hypertrophy. On the whole, acini regular; acinus cells of medium size, but in places slightly larger. Colloid usually solid, retracted, but there may be some vacuolar, adherent colloid.

Grade V. Normal gland without hypertrophy. Regular acini, low to medium epithelium. Solid, slightly retracted colloid.

Grade VI. Acini generally very small; epithelium very flat. Colloid solid and slightly retracted. There is some indication that acini have been dissolved.

A COMPARISON BETWEEN THE EFFECTS OF THE EXTIRPATION OF THE GREATER PART OF THE THYROID GLAND ON THE COMPENSATORY HYPERTROPHY OF THE REMAINING GLAND TISSUE IN GUINEA-PIGS WHICH RECEIVED KI AND IN CONTROL GUINEA-PIGS

I AND II KI SERIES.

I Series.* Twelve guinea-pigs were used. KI was injected subcutaneously; duration of individual experiment varied between seventeen and twenty-nine days. Average grade of hypertrophy was 3.54.

II Series.† Seven guinea-pigs were used. KI was fed by mouth; duration of

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* This series was previously reported, *J. Med. Res.*, 1920, xli, 481. One case is omitted here, because the animal had not received an injection during the last ten days.

† This series was also previously reported; one guinea-pig included in the earlier report has been here omitted, because the remaining gland tissue was removed as early as fourteen days after operation.

individual experiments was twenty-four to twenty-five days. Average grade of hypertrophy was 2.8.

The average grade of these nineteen experiments combined was 3.21.

In these two series, the experiments were carried out during the winter as well as during the spring months.

The weight of the guinea-pigs varied between 400 and 800 gm.

The result of the individual experiments was as follows: Grade of 1.75 in 3 animals; 2.25 in 2 animals; 2.50 in 1 animal; 2.75 in 1 animal; 3.0 in 2 animals; 3.25 in 1 animal; 3.75 in 4 animals; 4.0 in 2 animals; 4.75 in 1 animal; 5.0 in 2 animals.

I Control Series. Twelve guinea-pigs may serve as controls for the animals of the I and II KI Series. The control animals were examined nineteen to twenty-five days following the operation. This series includes 9 animals of a first lot with an average grade of hypertrophy of 4.50, and 3 animals of a second lot with an average grade of 3.50. The total average grade was 4.06.

The weight was approximately the same as in the KI series, namely, 400 to 800 gm. Individual grades: 3.25 in 2 animals; 3.75 in 2 animals; 4.0 in 5 animals; 4.75 in 1 animal; 5.0 in 2 animals.

III KI Series.

Sixteen guinea-pigs. Duration of individual experiments varied between eighteen and thirty-one days. This series of experiments was carried out in the winter and spring. The average grade of hypertrophy was 3.69. The weight of the guinea-pigs varied.

Individual grades: 1.50 in 1 animal; 2.25 in 1 animal; 2.50 in 1 animal; 2.75 in 1 animal; 3.0 in 1 animal; 3.25 in 1 animal; 3.50 in 2 animals; 4.25 in 3 animals; 4.50 in 2 animals; 4.75 in 2 animals; 5.25 in 1 animal.

Nineteen experiments of the *II Control Series* correspond to the III KI Series. The duration of the individual experiments varied between nineteen and thirty-two days. The average grade of hypertrophy was 4.14. Individual grades: 1.50 in 1 animal; 3.0 in 2 animals; 3.25 in 5 animals; 4.0 in 1 animal; 4.25 in 2 animals; 4.75 in 3 animals; 5.0 in 5 animals.

IV KI Series.

Ten guinea-pigs were fed KI during a period which varied in nine animals between fifty-two and sixty-four days. In one animal the experiment was terminated thirty-one days after operation. The average grade of hypertrophy was 3.40. The individual grades were as follows: 2.50 in 1 animal; 3.0 in 3 animals; 3.25 in 1 animal; 3.50 in 1 animal; 3.75 in 2 animals; 4.25 in 2 animals.

Nineteen animals, one constituting subseries (*b*) of the *IV Control Series*, correspond to this IV KI Series. The duration of the individual experiments varied between thirty and sixty-five days. The average grade of hypertrophy was 4.36. The individual grades were as follows: 2.75 in 1 animal; 3.25 in 1 animal; 3.75 in 2 animals; 4.0 in 3 animals; 4.25 in 1 animal; 4.50 in 1 animal; 4.75 in 5 animals; 4.80 in 1 animal; 5.0 in 4 animals.

In fourteen of these nineteen animals, the duration of the experiments varied between forty-two and sixty-five days. In this subseries the average grade of hypertrophy was 4.54. The individual grades were as follows: 2.75 in 1 animal; 3.75 in 1 animal; 4.0 in 1 animal; 4.50 in 1 animal; 4.75 in 5 animals; 4.80 in 1 animal; 5.0 in 4 animals.

V KI Series (1922–23).

Altogether ten animals were fed KI in this series. The average weight of guinea-pigs varied between 400 and 600 gm. The duration of the individual experiments varied between thirty and thirty-two days. About one-fourth to one-fifth of one lobe was left at the operation. There were six experiments done during the summer months with an average grade of hypertrophy of 4.4 and four winter experiments with an average grade of hypertrophy 4.0. The total average was 4.25. The individual grades of the summer experiments were: 3.50, 3.75, 4.50 and 4.75, each in 1 animal; 5.0 in 2 animals. In the four winter experiments the individual grades were: 3.50 in 2 animals; 4.25 in 1 animal.

The following experiment may be cited as an example: guinea-pig 94, one lobe of thyroid completely removed and the greater part of the second lobe extirpated. Daily feeding of KI, 0.05 gm. Thirty days later animal killed by chloroform and examined. Weight of animal at time of operation was 564 gm.; at time of examination 607 gm. At necropsy about one-third of one lobe of thyroid but no isthmus was found. Microscopic examination shows acini to be irregular in outline; some acini are long drawn out. Through the solution and absorption of the walls separating neighboring acini some papillae are produced. The acinus cells are somewhat enlarged. The colloid is pale, partly vacuolar, partly absorbed; the quantity is reduced. Some acini are infiltrated with lymphocytes and some polymorphonuclear leucocytes and these cells contribute to the absorption of the colloid. The grade of hypertrophy was 3.50.

To this series corresponds the *VII Control Series* with twelve experiments. Eight of these were winter and four summer experiments. The duration of the individual experiments was thirty days. The average hypertrophy of the summer experiments was 4.4 and of the winter experiments 4.25. The total average was 4.3. The individual grades were: 3.25 in 1 animal; 3.75 in 2 animals; 4.0 in 2 animals; 4.25 in 1 animal; 4.50 in 1 animal; 4.75 in 1 animal; 5.0 in 4 animals. The grades in the four winter experiments were 3.75, 4.0, 4.25 and 5.0. In this series the difference between the KI Series and the Control Series is very slight.

VI KI Series. Winter, 1924 (January to March).

Eight experiments were carried out. Average weight of the guinea-pigs 400 to 600 gm. One-fourth to one-fifth of one lobe of the thyroid was left at the operation. Duration of individual experiments thirty to thirty-one days. At examination one-fourth to one-half lobe is found. Average grade of hypertrophy is 3.40. Individual grades: 2.25 in 1 animal; 2.75 in 1 animal; 3.0 in 1 animal; 3.25 in 2 animals; 3.75 in 1 animal; 4.75 in 2 animals.

The following is an example of a decided hypertrophy in a guinea-pig of the KI series: guinea-pig 453, male, 642 gm. initial weight

and 647 gm. end weight. Duration of experiment thirty-one days. At examination, one large and two small remnants, altogether more than one-third of a lobe was found. No isthmus. Microscopic examination: very irregular, large papillary acini with high epithelium. Some mitoses in acinus cells. Very much diminished, softened, partly liquefied colloid; in places it has entirely disappeared. In some acini the colloid is filled with cells; some phagocytes in acini. Some acini form only slits. Through degeneration (pyknosis) and solution, walls of acini disappear. Still some papillae are visible which are composed of cells with pyknotic nuclei and which are approaching solution. Large conglomerate acini with multiple papillae may develop. Grade of hypertrophy 2.25.

The *VIII Control Series* of seven animals corresponds to this series. The average grade of hypertrophy in this series was 4.21. The individual grades were as follows: 3.25 in 2 animals; 4.25 in 2 animals; 4.75 in 2 animals; 5.0 in 1 animal.

VII KI Series (June and July, 1924).

Nine experiments were made in which one-fourth to one-fifth of each lobe was left and subsequently 0.1 gm. KI was fed daily to each of five guinea-pigs and one-fourth of this amount to each of four other guinea-pigs. The initial weight of the guinea-pigs varied between 450 and 600 gm. The duration of each experiment was twenty-nine days. The average grade of hypertrophy was 4.27. The individual grades were as follows: 3.50 in 1 animal; 3.75 in 2 animals; 4.0 in 1 animal; 4.25 in 1 animal; 4.75 in 3 animals; 5.0 in 1 animal.

The findings in guinea-pig 52 (a female from 583 gm. initial weight to 664 gm. end weight; fed 0.025 gm. KI daily) were as follows: two remnants of thyroid were found twenty-nine days after operation; no isthmus. In piece "a" the acini show slight irregularities; some papillae are found; some large acini. The acinus cells and nuclei are hypertrophic. There are many mitoses. In these acini the colloid is soft, often vacuolar or partly dissolved. Some phagocytes in colloid take up some of the colloid. In places the colloid is somewhat retracted. The connective tissue is edematous. Piece "b" is on the whole similar. The acinus cells are here slightly enlarged, and the colloid vacuolar and soft. Frequent mitoses. Irregular acini. There are areas where the epithelium shows no enlargement and the colloid is solid, retracted. Grade of hypertrophy 3.75.

The *IX Control Series* corresponds to this KI Series. The time, duration of experiments, mode of operation and weight of animals were approximately the

same, but the guinea-pigs did not receive KI after the partial extirpation of the thyroid gland. Seven experiments were made; the average grade of hypertrophy was 4.80. The individual grades were as follows: 4.75 in 5 animals; 5.0 in 2 animals.

If we compare the average grade of hypertrophy in each of the seven KI series and control series, we find in each instance the average of the hypertrophy more marked in the KI series, except in the V Series where the six experiments of the KI series and the four summer experiments of the control series showed the same, relatively low grade of hypertrophy.

KI Series	Control Series
I Series — 12 guinea-pigs; subcutaneous injection of KI; 17–29 days. Average grade of hypertrophy 3.54.	I Control Series — 9 guinea-pigs; average grade 4.50. Three guinea-pigs; average grade 3.50. Average grade of both combined 4.06.
II Series — 7 guinea-pigs; KI fed by mouth; 24–25 days. Average grade of hypertrophy 2.8. Average grade of Series I and II combined 3.21.	
III KI Series — 16 guinea-pigs; 18–31 days. Average grade of hypertrophy 3.69.	II Control Series — 19 guinea-pigs; 19–32 days. Average grade of hypertrophy 4.14.
IV KI Series — 10 guinea-pigs; 52–64 days (1 animal 31 days). Average grade of hypertrophy 3.40.	Subseries "b" of IV Control Series — In 14 guinea-pigs average duration of experiment varied between 42 and 65 days. Average grade of hypertrophy 4.54. In 5 additional animals of this series the duration of the experiment was above 30 days, but not as long as in the 14 animals. The average grade was 3.85. The grade of both lots combined 4.36.
V KI Series — 10 guinea-pigs; duration 30–32 days; 6 summer experiments, grade 4.4; and 4 winter experiments, grade 4. Total average of hypertrophy 4.25.	VII Control Series — 12 guinea-pigs; duration 30 days; 8 winter experiments; 4 summer experiments. Average grade of hypertrophy in summer experiments 4.4; in winter experiments 4.25. The average of both combined 4.3.
VI KI Series, winter, 1924 — 8 guinea-pigs. Duration 30–31 days. Average grade of hypertrophy 3.40.	VIII Control Series — 7 guinea-pigs; same duration as KI Series. Average grade of hypertrophy 4.21.
VII KI Series, summer, 1924 — 9 guinea-pigs. Duration 29 days. Average grade of hypertrophy 4.27.	IX Control Series — 7 guinea-pigs. Average grade of hypertrophy 4.80.

If we compare the average grade of hypertrophy of all the KI guinea-pigs with the average grade of all the control guinea-pigs, we find the following figures: Altogether seventy-two animals of the KI series lived until the completion of the experiments. The average grade of hypertrophy in these seventy-two animals is 3.65. Seventy-six guinea-pigs are included in the control series; their average grade of hypertrophy is 4.24. If we enlarge our number of control animals to 117 by including guinea-pigs in which the greater part of the thyroid gland had been removed, but in which no special substance was fed and which served as controls in other experiments, we find an average grade of hypertrophy of 4.13; this figure still differs distinctly from the figure applying to the KI series. This figure confirms the conclusion based on a comparison of the KI animals with their proper controls.

However, a consideration of the averages does not provide an entirely adequate basis of comparison of both series. For certain reasons of which we are at present ignorant, the grade of hypertrophy varies considerably in different individuals and within the same series. Furthermore, our grades are to some extent arbitrary and not expressing the differences between various animals in an entirely adequate manner. Thus the difference between 1.0 and 3.50 according to our grading is greater than the difference between 3.75 and 5.0; yet in certain respects the difference is perhaps greater between the two latter than between the two former animals. Five represents absence of hypertrophy. On the other hand, 1.0 and 3.50 merely signify different intensities of hypertrophy. It will therefore be necessary to supplement the average grades with the comparison of the number of animals showing the various grades of hypertrophy in the KI and in the control series.

GRADES OF HYPERTROPHY

	KI Series	Control Series
Grade:	1 — 2 (excl.) : 4 guinea-pigs	1 guinea-pig
"	2 — 3 (excl.) : 10 guinea-pigs	1 guinea-pig
"	3 — 4 (excl.) : 29 guinea-pigs	19 guinea-pigs
"	4 — 5 (excl.) : 22 guinea-pigs	37 guinea-pigs
"	5 or more : 7 guinea-pigs	18 guinea-pigs

We can consider the grades 4.50 and higher as indicating lack of hypertrophy, the grades 3.50 (incl.) to 4.50 (excl.) as indicating a

mild degree of hypertrophy and the grades with a lower figure than 3.50 as indicating a very distinct and marked hypertrophy. We thus have three classes of animals representing different intensities of hypertrophy.

KI Series

Class I	(no hypertrophy)	: 19 guinea-pigs = 26 per cent	= 26 per cent
Class II	(mild hypertrophy)	: 27 guinea-pigs = 38 per cent	with hypertrophy 74 per cent
Class III	(marked hypertrophy)	: 26 guinea-pigs = 36 per cent	

Control Series

Class I	38 guinea-pigs = ...	50 per cent
Class II	23 guinea-pigs = 30.3 per cent	with hypertrophy 50 per cent
Class III	15 guinea-pigs = 19.7 per cent	

The comparison of the number of animals in the various classes in the KI series and control series shows a decided increase in the hypertrophy in the KI series over the control series.

Discussion

The results which we obtained in these experiments make definite the conclusion that potassium iodid does not have any inhibiting effect on the hypertrophy which develops in the guinea-pig in the remaining part of the thyroid gland after the greater part has been removed. On the contrary, they make it almost certain that KI intensifies the microscopic characteristics of hypertrophy during the period from the twentieth to the sixtieth day following extirpation. It increases the average size of the acinus cells and the number of mitoses; it promotes the liquefaction and absorption of the colloid; and tends to make the acini more irregular. As far as the macroscopic appearance is concerned, the remnants of the gland in the KI series are usually of good size.

We shall now attempt to correlate in a tentative way the principal facts established concerning the relations of iodin and the thyroid gland under pathologic conditions. In the guinea-pig we find that after extirpation of a great part of the thyroid, compensatory hypertrophy sets in in many cases and that this leads to a more or less far-going transformation in the structure of the gland. Thyroid feeding prevents hypertrophy and may perhaps even lead to a relative atrophy of the gland; feeding anterior lobe of the pituitary

also prevents the compensatory hypertrophy without apparently leading to an atrophy of the gland; the feeding of thymus has no noticeable effect; and the feeding of potassium iodid or the administration of iodin in some other form does not only not prevent or diminish the hypertrophy, but on the contrary tends probably to increase it.

However, we find that there are variable factors in the compensatory hypertrophy which are understood only very incompletely at the present time. A considerable number of guinea-pigs do not show these signs of hypertrophy which we find in other cases. Seasonal differences in weather seem to play a rôle in these variations, but they do not account for differences found in animals operated upon during the winter months. It may be that the time curve varies in different animals, and that in some of them the changes take a more rapid course than in others. In particular, it is thus possible that in control animals the hypertrophy takes a more rapid course than in the KI animals and that in the earlier stages which still have to be investigated, the relative preponderance of signs of hypertrophy is not, as at later periods, on the side of the KI guinea-pigs but rather on that of the controls. It may also be that in some animals a compensatory new formation of acini develops without the signs of hypertrophy occurring. However, the occurrence of mitoses in the acinus cells is usually associated with at least some hypertrophy of the gland cells. All these points need further elucidation before the problem of compensatory hypertrophy can be considered completely solved.

If we now turn to goiter in man, iodin seems to have different effects under different conditions. In children, small doses of iodin taken over a long period of time seem to prevent the endemic goiter, at least the kind that occurs in the Great Lakes region in this country. It also seems to cause the retrogression of developing goiter in young children; whether it has the same effect in Switzerland in the case of endemic goiter frequently associated with cretinism, needs still to be established. In the case of goiter of the adult, iodin does not seem to lead usually to a complete retrogression of the goiter. At most it seems to cause a temporary retrogression which is in many cases followed later by new growth. It has also been observed that a goiter which diminished in size as a result of a first administration of iodin, is in some cases found refractory to the

action of iodin if it is applied again at a later period. But in addition, iodin apparently causes in many cases a transformation of a simple goiter into a condition which, as far as structure of the gland and clinical symptoms are concerned, closely approaches Graves' disease. This transformation takes place especially in nodular goiters, but it may also occur in the case of the diffuse goiter.

In Graves' disease the administration of small doses of iodin seems to diminish the severity of the symptoms at least for a short time; it does not lead to a cure. As soon as the intake of iodin is interrupted the symptoms occur again. And even with long continued administration of iodin the symptoms seem to return after a transitory stage of improvement, although it has been stated by Fraser that a diminution in the doses of iodin may lead to a longer continued remission in the symptoms. In the case of toxic nodular adenoma of the thyroid gland, administration of iodin appears to aggravate the symptoms.

These seem to be the principal effects of the administration of iodin as far as they have been established at the present time. These various data appear in certain respects contradictory. The following attempt to interpret them can be regarded only as purely tentative and will have to be modified in accordance with the accumulation of additional experimental and clinical data.

The diminution of the specific substance (hormone) normally produced by the thyroid gland initiates changes in the gland, or perhaps primarily in the organism as a whole, which lead to hyperactivity and hypertrophy of the thyroid and thus tend gradually to counteract more and more through an increased production and discharge of hormone, the causes which led to hypertrophy. Thus, the lack of the specific hormone acts as a stimulus to the gland. If the hormone is administered directly the changes leading to thyroid hypertrophy are removed.[2] A somewhat related substance in the anterior pituitary gland acts in a similar, but not entirely identical way.[3] In order to undergo this compensatory hypertrophy iodin is needed and the gland tissue in process of hypertrophy has the ability to bind this substance and to use it for the building up of hormone and also of the hypertrophic gland cells. There is usually a sufficient amount of iodin taken in with the food to permit this restitutive process (hypertrophy) to proceed; but the optimal quantity does not seem to be present in many cases and therefore administration

of an additional amount of iodin increases the intensity of hypertrophy in many animals. Not before the organism has obtained a sufficient amount of hormone, does the stimulating mechanism come to a standstill. This point seems to be reached a long time before the remaining part of the thyroid has rebuilt the whole amount of thyroid removed. According to our experiments the hypertrophy of the thyroid gland in the guinea-pig is thus never complete, at least in the time during which we kept the animals under observation. Thus we may explain the difference which we found in the effect of thyroid or pituitary substance on the one hand and of potassium iodid on the other, the two former suppressing or inhibiting hypertrophy, while the latter on the contrary stimulates it.

If in infants and young children the amount of iodin necessary for the production of the hormone is lacking, growth processes set in in the thyroid gland, which are different from those characteristic of compensatory hypertrophy and Graves' disease, and are more nearly akin to those growth processes which lead to the embryonal development of the thyroid. Again we assume that not only in order to build up hormones, but also in order to undergo those specific growth processes consisting in hypertrophy of the acinus cells, combined with absorption of colloid, a certain minimum amount of iodin is required and that in the absence of this amount of iodin the growth processes occurring in the thyroid gland in response to the deficiency in hormone are of a different kind, consisting essentially of a new formation of acini, which are in a relative state of rest. The epithelium is therefore relatively low, the acini are regular and colloid is stored in solid form. This may be considered a sparing reaction on the part of the thyroid gland in which the small amount of hormone produced is stored in the acini where it can be slowly and gradually used, thus compensating for the diminished facilities for producing this necessary material for lack of which the gland suffers. On the other hand, in compensatory hypertrophy and in Graves' disease, the gland works with full vigor, gives off at once the hormone produced and thus, at least in the case of compensatory hypertrophy, it attempts to compensate for a deficient amount of substance by the increased intensity with which the remainder is used. Both of these reactions may therefore be considered as adaptations of the thyroid gland to the specific abnormal condition in which it is placed.

Furthermore, in compensatory hypertrophy the gland not only produces hormone in a relatively increased quantity, but it builds up increased amounts of thyroid tissue and thus gradually diminishes the need and with it the stimulus for increased activity. In contrast to this condition, the growth processes in the developing goiter in children cannot markedly lead to an increase in the production of hormone because the supply of iodin necessary for the building up of hormone is not provided.

If at this stage an additional amount of iodin is furnished the gland, it returns to its normal active state because the additional iodin removes the deficit in hormone and the gland can begin to function under adequate conditions, provided lack of iodin was the only or at least the principal factor in the causation of the goiter. If other important factors should contribute to or be mainly responsible for the development of goiter, it is conceivable that administration of iodin may not have the same restorative effect. But if this deficit in iodin continues for a long period of time, it is conceivable that changes gradually occur in the cell equilibrium which lead to abnormalities, in so far as (1) the growth of the gland may become irregular and certain parts may proliferate more than others, so that a nodular goiter develops in which again an increased amount of colloid may be stored with the consequent formation of a colloid goiter; and, (2) in addition, the equilibrium of the acinus cells changes in such a way that the reaction to iodin becomes different.

We know that under other conditions changes of a somewhat similar character may take place as a result of preceding stimulation in living organisms; a change perhaps somewhat akin to active cellular immunity or allergy. If after the developmental period of the thyroid has passed iodin is furnished to the gland, it no longer responds to it in the manner in which it did at an earlier stage. It has become either entirely or almost entirely refractory to iodin which is now no longer able to lead to the normal growth restriction as a result of adequate hormone production. In other words the gland has lost the ability to produce the normal amount of hormone even in the presence of iodin. In some cases these structural changes may perhaps be very slight or may be limited as yet to small areas so that they may escape recognition. The increased amount of hormone is given off in such cases in so large a quantity and so rapidly that toxic symptoms may develop. Similar is the effect

which has been observed after administration of iodin in cases of thyroid adenoma. The gland tissue is no longer under hormonic regulation to the same extent as is the normal gland or the early goitrous gland in children, a condition which bears some analogy to the behavior of tumors which also are only incompletely or not at all under hormonal or metabolic regulation on the part of the organism as a whole; while this is especially so in the case of malignant tumors, it is also found, although to a less extent, in the case of adenomas.

In the case of Graves' disease we have to deal also with a gland which apparently is no longer under the complete control of the organism. It over-provides the hormone and discharges the substance rapidly into the circulation. It thus behaves somewhat like a gland in compensatory hypertrophy, and the structure of the gland fully corresponds to this increased activity. We may, therefore, assume that in this condition also, the gland is in a stimulated state; but the character of the stimulation which leads to the increase of activity in the two cases may differ. It is unknown in the case of Graves' disease. In the latter disease, it is not due to absence of a sufficient quantity of thyroid tissue. While in compensatory hypertrophy the amount of active gland is much reduced and therefore toxic symptoms are lacking, in the case of Graves' disease the quantity of the active gland is not only not decreased, but increased, and thus toxic symptoms are produced. In both compensatory hypertrophy and Graves' disease there is the normal amount of iodin present in the organism and therefore the gland can undergo the same structural changes in both cases. Yet a relative lack of iodin may exist in Graves' disease, considering the increased amount of thyroid tissue and the excessive activity of the gland in this disease. Even in the case of compensatory hypertrophy, we found that administration of iodin in certain cases tended to increase the activity of the remaining gland, thus indicating a relative lack of iodin. We may, therefore, assume that in Graves' disease the optimal amount of iodin satisfactory for a hyperactive gland is not at hand; that this lack may either, in accordance with the suggestion of Plummer, lead to the formation of abnormal products which cause symptoms of intoxication, or may still further stimulate the gland to abnormal activity; and that the administration of iodin may remove at least some of the abnormal conditions under which the gland functions, thus diminishing temporarily the overactivity

of the gland. However, other interpretations of this effect are possible. At present it seems that no definite statement can be made as to the manner in which iodin diminishes the toxic symptoms in Graves' disease.

Conclusion

On the basis of a very large number of experiments it can be definitely stated that potassium iodid does not prevent or even diminish the hypertrophy of the thyroid gland in the guinea-pig, which follows extirpation of a great part of the thyroid gland. On the contrary, in all our series of experiments, the average of hypertrophy was greater in the animals which received potassium iodid than in the control animals. There are, in addition to the amount of thyroid removed and the amount of iodin fed to the animals, other variable factors which influence the degree of hypertrophy following extirpation, such as seasonal changes. As in former experiments, the average hypertrophy was less marked in the summer months than that in experiments carried out during the winter. There are other variable factors which influence the result, which are as yet unknown.

On the basis of these results a tentative explanation of the influence of iodin on the structure and function of the thyroid gland is given.

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CONCERNING "MALIGNANT THYMOMA"; WITH A REPORT ON A CASE OF PRIMARY CARCINOMA OF THE THYMUS *

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Introduction

Since reporting a case of malignant tumor of the thymus five years ago (Foot[1]), I have studied and reported two more; a fourth recently examined at necropsy in our department will serve as a basis for this paper and afford the opportunity for discussing malignant tumors of the thymus as a group. None of the four cases in this series exactly resembles any of the others, excepting that two were of the sarcomatous type, one being large-celled and the other small-celled. A review of this short series, which makes possible a critical and comparative study, has so changed my views on the histogenesis and classification of this type of neoplasm that a discussion of these seems desirable, as an attempt to clear up some of the confusion that exists concerning the "malignant thymoma." After reporting this fourth case, therefore, the entire subject will be considered at some length.

These tumors are distinctly rare, less than a hundred having been described to date. The epithelial type of thymoma is still rarer, Symmers and Vance[2] in 1921 stating that only four true primary carcinomas of the thymus had been reported prior to that time. Since then Foot and Harrington[3] and Jacobson[4] have each described one. As the recent average has been less than two cases of malignant thymic tumor reported annually, I have been unusually favored in being able to collect four cases in the last five years.

Case Report

Clinical Report. F. H., a white male, 45 years old, single and a gasfitter by occupation, was admitted to the Cincinnati General Hospital on four occasions between February 13 and May 30, 1925, when he was discharged dead.

Chief Complaint. Shortness of breath and pain in the chest, with cough.

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Present Illness. He felt well until nine weeks ago, when he began to have a dry cough which was brought on and intensified by stooping over. This attitude also caused dyspnea, weakness and dizziness. Shortly after the cough began he noticed swelling of his neck, most marked just after a fit of coughing, when his face became puffy. These symptoms increased until he was forced to prop himself up on a pillow in order to sleep in bed. He noted burning sensations and pain beneath the sternum, extending to the back, across the right chest and localizing between the scapulae. His appetite was good, his bowels regular and he had had no nausea or vomiting.

Past History. His general health had always been good. Aside from a history of chancroid and bubo nine years ago, his past history is irrelevant.

Family History. There was no history of malignant tumor in his family and no data were obtained that bear on this case.

Physical Examination. Negative, except for the chest and neck. There was engorgement of the veins over the right arm and in the right axilla. The respiratory movements were free and equal. The percussion note was impaired over the right upper chest, slightly so above and behind on the right. The breath-sounds were diminished over these areas. The neck was full; there was a firm mass beneath the right clavicle, extending upward and backward behind its outer half. The cricoid cartilage was at the level of the suprasternal notch. The thyroid was not palpable, but its isthmus seemed to impinge upon the examining finger during inspiration. There was no tracheal tug. The retroclavicular mass did not pulsate. Retrosternal dullness was markedly increased, being 8.5 cm. in the first interspace, 3.2 and 5.0 cm. in the second. Cardiac dullness was 2.5 cm. to the right, 12 cm. to the left; the point of maximum impulse was 10 cm. to the left in the fifth interspace.

Subsequent Course. The venous engorgement increased and the breath-sounds and percussion note became progressively impaired as time passed. Two weeks after admission, the patient complained of feeling a mass that rose and fell behind his sternum with each respiration. His cough persisted. He was given deep radiotherapy at the Cincinnati Jewish Hospital and, as the diagnosis of malignant mediastinal tumor was practically certain, he was told to continue this treatment once a week and to report frequently for observation. A skiagraph showed kyphoscoliosis and a large shadow in the mediastinum. Laboratory tests at this time were all negative.

On his first readmission the condition was found to be getting progressively worse; despite the weekly irradiation with deep X-rays there was more bulging in the right supraclavicular fossa and some had appeared in the left. His voice had become husky, but his cough was less troublesome. There was more fullness in the upper chest and neck and the mediastinal growth was unmistakably increasing. Nausea and vomiting were now added to the list of symptoms.

On his second readmission the patient remained only a day or two. In the two months that intervened since his first admission, the mass in the mediastinum had grown steadily and the superficial abdominal veins now shared in the engorgement hitherto noted over the upper chest and right arm. His cough had reappeared and he had developed an early secondary anemia.

On his third readmission he remained two weeks in the hospital and died about three and one-half months after the date of his first admission. His gastroenteric symptoms had increased; there was definite orthopnea; and the swelling in the neck had extended well upward beneath the right sterno-mastoid.

The superficial veins were now generally distended, particularly over the lower thorax. A wide area of retrosternal dullness was determined 4.5 cm. to the right and 5.5 cm. to the left in the fifth interspace. Dysphagia had developed. His pupils and pulses remained equal on both sides. Two weeks after his last admission, his cough which had been persistently dry and unproductive became productive and he raised large quantities of slaty, homogeneous sputum. Two days later, after feeling very ill for a day or so, he had a sudden profuse hemorrhage that gushed from his nose and mouth and he died within four or five minutes. During his final visit to the hospital his temperature, which had hitherto been essentially normal, became febrile and ranged from 99 to 102 F. Two skiagraphs showed that the tumor had increased until it had involved the entire upper lobe of the right lung. His treatment was, of necessity, symptomatic: deep X-ray therapy once a week and codeine to control his cough were the chief measures used.

NECROPSY. This was performed forty-five hours postmortem by Dr. H. A. Day, Resident in Pathology.

Anatomic Diagnoses: Carcinoma of the mediastinum with metastasis to both lungs, parietal pleura and to the peribronchial, supraclavicular and right axillary lymph nodes. Necrosis of the upper lobe of the right lung with fatal erosion into the right bronchus and its vessels. Extensive hemorrhage into right bronchus. Lobular pneumonia of right middle and lower lobes; also of the left lung, with extensive aspiration of fresh blood and possible hemorrhages. Healed fibrous pleuritis, right hydrothorax. Obliteration of the superior vena cava by ingrowth of the tumor. Brown atrophy of the heart and chronic passive congestion of the liver, spleen and kidneys. Healed cholecystitis and peritonitis. Fibrosis of the spleen and prostate. Subconjunctival hemorrhages, Residental caries and pyorrhea alveolaris.

Anatomic Findings: Body. That of a fairly well developed and somewhat emaciated white man apparently between fifty and sixty years of age. Body length 163 cm. Rigor mortis is universally present and there is slight postmortem lividity in the dependent portions. The hair is gray, the scalp rather bald. A bloody discharge escapes from the nose and mouth. The corneae are clear and the pupils unequal: right, 3 mm., left, 2 mm. in diameter. There are several petechial subconjunctival hemorrhages in both eyes. The mouth is befouled and coated with dried blood; the teeth are almost all gone, those remaining are mere snags and the gums are retracted and suppurating. The superficial lymph nodes are palpable as discrete nodules, excepting those of the right axilla and the supraclavicular group which are considerably enlarged and very firm. The extremities are all edematous, the right more so than the left.

Primary incision from the suprasternal notch to the mons pubis, with lateral subclavicular extensions, reveals very little subcutaneous fat. The peritoneal cavity is smooth, gray and blood-tinged and contains a few cubic centimeters of bloody, serous fluid. There are a few fibrous adhesions between the liver and diaphragm and between various intestinal coils, the colon and the gall bladder.

Thorax. Upon removing the sternum, a large mass is at once seen in the mediastinum, more or less adherent to the sternum and situated chiefly in the anterior mediastinum, where it surrounds the great vessels and adheres to the pleurae of both lungs. It is grayish, hard and composed of much fibrous tissue which cuts with a leathery resistance. It extends from just above the clavicles

to more than half way to the diaphragm and has included the mediastinal lymph nodes in its substance. These have a softer, yellower appearance quite different from that of the tumor, and several of them are necrotic and caseous. The necrosis resembles that of tuberculosis rather than that of tumor tissue. The neoplasm has invaded and completely occluded the superior vena cava and infiltrated the adventitia of the large arteries without penetrating their media. This infiltration has caused a definite constriction of the innominate artery, but the left subclavian and carotid do not seem to be much compressed although completely surrounded. The left pleural cavity is entirely obliterated by fibrous adhesions and is much thickened and apparently infiltrated by the tumor at its vault; the right pleural cavity is also obliterated in its upper portion by similar adhesions, but contains about 500 cc. of blood-tinged fluid in the lower. The heart is not remarkable.

The pleura of the right lung is covered with adhesions and dotted with gray tumor metastases which are more numerous over the upper lobe and somewhat umbilicated where they spread out beneath the pleura. On section, the upper lobe is found to contain a cavity 3×4 cm., in its approximate center; the walls of this cavity are necrotic and that of the right bronchus has been eroded and perforated just below the tracheal bifurcation. The tracheal and bronchial mucosa is eroded and congested in a zone 3 cm. in width, surrounding the perforation. Masses of firm, not yet necrotic tumor tissue lie in the walls of the pulmonary cavity and a few metastases are scattered throughout the rest of the lobe. The pulmonary tissue is also the seat of a patchy consolidation. It cuts with considerable resistance, is grayish and mottled with areas of congestion and anthracosis. The lower lobes are both congested and contain a few tumor nodules. The left lung is covered with shaggy, fibrous pleural adhesions throughout. On section it is found to contain numerous small areas of consolidation and congestion and a few minute tumor metastases. The bronchioles all contain fresh blood, probably aspirated.

The trachea is surrounded by the tumor as high as the suprasternal notch; its mucosa is edematous and congested. The bronchi contain fresh blood clots and the right shows the perforating erosion already described. The peribronchial lymph nodes are incorporated in the neoplasm and show anthracosis, caseation and a few areas suggesting metastases. The thyroid is not remarkable in gross, except for a small node in the anterior lower aspect of the right lobe. This later proves to be a parathyroid.

Abdomen. The abdominal organs show nothing relevant to this paper. The spleen weighs 190 gm., the liver 1655 gm. and the kidneys together weigh 315 gm. No extension of the tumor below the diaphragm is noted.

Microscopic Study. Diagnoses (N. C. F.): Primary carcinoma of the thymus with metastases to lungs, vessel walls and lymph nodes. Cardiac edema and increase in size of myocardial fibers. Low-grade lobular pneumonia with much organization of the exudate; interstitial pulmonary fibrosis. Infiltration of tracheal and aortic walls by the tumor. Fibrosis of the spleen; fibrous perisplenitis. Moderate toxic nephritis. Chronic fibrous prostatitis and slight subacute suppurative prostatitis.

Examination: The findings in the sections of various organs correspond to the above diagnoses and will not be dwelt upon, excepting where they have a direct bearing on the tumor. The lung sections show tumor metastases in which the neoplasm invades the alveoli, growing luxuriantly and producing innumer-

able Hassal's corpuscles. The wall of the trachea is infiltrated throughout by tumor tissue which has penetrated to the lumen and separated and destroyed the cartilage in so doing.

Microscopic Examination of the Tumor

Sections from the primary growth in the mediastinum are of comparatively little value for study, as there is so much necrosis that the tumor has been converted into dense masses of interlacing connective tissue interspersed with wide necrotic areas in which the outlines of many very large concentrically laminated bodies are evident (Fig. 1). There are wavy columns of young, well stained tumor cells at the periphery of necrotic areas. There is a widely distributed network of reticulum in this necrotic tissue. Undoubtedly the repeated irradiation with deep X-rays played a part in causing this breaking down of the tumor.

The neoplasm is best studied in its metastases to the lung and lymph nodes where there is far less necrosis and one can make out the morphology of the type cell. This corresponds to the thymic reticuloid cell and is about 10 to 17 microns wide and 13.5 to 34 microns long, according to its type of growth and stage of development (Figs. 2 and 3). The nucleus is elliptical and measures 6.5 to 13.5 microns in width and 10 to 17 microns in length. Three types of growth may be recognized: (*a*) solid, alveolar masses that resemble those of a medullary carcinoma, sometimes suggesting gland formation about rudimentary lumina; (*b*) anastomosing stellate cells with far less cytoplasm than the preceding type, forming alveoli of loosely reticulated structure; and (*c*) keratinized agglomerations of a few to many concentrically arranged cells identical in their appearance with Hassal's corpuscles.

The nuclei of the first two types are more or less vesicular, with a distinct chromatin network of delicate but rather deeply staining threads; one or more nucleoli are visible in each nucleus. The nuclei of the third type are usually rather pyknotic and degenerated. The cytoplasm of the tumor cells varies from scanty and vacuolated to very dense and refractile, resembling that of glandular epithelium and of keratinized dermal epithelium in the latter case.

The stroma is very fibrous and abundant in the primary growth and is composed chiefly of collagenous connective tissue; in the younger portions of the tumor and in the fresh metastases it is al-

most exclusively made up of reticulum. This surrounds the alveoli of the tumor without penetrating them (Fig. 4). The stellate type of tumor cell forms a reticulated matrix within the alveoli and this does not become impregnated with silver, being distinct from the true reticulum of the stroma and composed entirely of cytoplasmic processes. The pulmonary and lymphoid reticulum is very abundant and coarse in the vicinity of the metastatic cells, but it stops short at the margin of the tumor alveoli without penetrating them, just as would be true of an alveolar carcinoma.

To summarize, then: the tumor is of a definitely epithelial type; its cells correspond to those of a loosely built alveolar carcinoma, but differ from them inasmuch as they are stellate and more like embryonal thymic reticulum cells and also because they tend to form bodies that resemble thymic corpuscles more closely than the epithelial "pearls" of an epidermoid carcinoma.

The formation of these bodies indicates that they are, indeed, degeneration products. At first a few cells are observed lying singly and staining more deeply than their fellows. They are more compact, appear somewhat shrunken and their nuclei become increasingly pyknotic, elongated and narrowed (Fig. 5). Next, one finds several such cells grouped together and when a number have become cornified and clumped, they show a concentric arrangement (Figs. 6 and 7). Finally, the centers of these whorls undergo caseation. It is very evident that there are more of these bodies in the degenerated portions of the tumor and that the large necrotic foci contain innumerable shadowy Hassal's corpuscles; sometimes one of the smaller fields appears to be composed of a single, gigantic thymic corpuscle.

Giant cells and endothelial phagocytes are attracted to the necrotic areas in some cases, but the polymorphonuclear leucocytes, so common in the Hassal's bodies of the involuting thymus, are notably scarce. Eosinophiles and lymphocytes are not component parts of this tumor; it appears to be purely epithelial.

Discussion

Development of the Thymus. This organ arises from a paired primordium developing from the entoderm of the third pharyngeal pouches in the form of two tubular downgrowths. Schäfer (Jacob-

son cit.) considers that the fourth pair of pouches may share in the production of the gland, but Prentiss and Arey [5] point out that this portion of the primordium atrophies and contributes little thereto. The tubular processes soon lose their lumina and become solid cords which swell below and atrophy above, thus forming the two thymic lobes. The upper portions may give rise to accessory thymic organs. While entirely entodermal in man, the thymus of moles is ectodermal and that of pigs and fowls of mixed origin. In the human 50 mm. embryo the thymus still contains solid cords and vesicles of entodermal origin, but soon after this takes on its well known lymphoid appearance. The supporting tissue of the gland is formed of large, branching cells of entodermal origin, some of which become compacted and keratinized to form Hassal's corpuscles which characterize the organ histologically. Marine (Prentiss and Arey cit.) believes that they are the hyalinized and atrophic remains of entodermal vesicles.

While the reticuloid cells and Hassal's corpuscles are of undoubted entodermal origin, the lymphoid cells and the numerous eosinophile elements are of disputed parentage. Controversy has centered upon the lymphoid elements: are they differentiated entodermal cells, or are they lymphocytes of mesodermal origin, which have migrated into the thymic cortex from without? The adherents to the former theory constitute the "monistic school," to which belong Bell, Prenant, Schridde and formerly Pappenheimer.[6] Pappenheimer [7] has changed his views on this subject during the last few years, as have I. The second theory is championed by the "dualists": Dantchakoff,[8] Maximow[9,10] and Hammar[11,12,13] who has probably done more original work on this organ than has anyone, as is attested to by his article "Fünfzig Jahre Thymusforschung." [12] That these lymphoid cells are really lymphocytes was clearly indicated, if not proved, by the thymic tumor reported by Friedlander and myself [14] last winter (1925), in which the neoplasm was lymphoid in type and accompanied by an unmistakable lymphoid leukemia without any noticeable involvement of the lymph nodes in general. Fabian (Ewing cit.) has already collected a small series of cases that show the same or similar blood pictures. Thus it seems very probable that the "dualists" are warranted in their assumption that these are true lymphocytes and not differentiated entodermal cells.

The question as to whether the eosinophile elements of the thymus are of entodermal or of blood origin is not entirely relevant to this discussion; moreover it is far from being settled.

Literature. The average text-book on pathology passes by the malignant thymic tumors with rather cautious brevity, but Ewing [15] has discussed them at length in his book on neoplastic diseases. Rubaschow [16] compiled a list of thymic tumors including a compilation made by Hoffmann in 1896 and found that 52 sarcomas, 12 carcinomas and 11 miscellaneous tumors had been described up to that time (1911). The reader will find these earlier cases listed in Rubaschow's excellent article. If we exclude the miscellaneous tumors and add those reported since 1911, a total of 81 reports will be obtained. Symmers and Vance [2] state that only four true primary malignant epithelial tumors have been reported up to the time they published their paper; since then Jacobson [4] has described one, Foot and Harrington [3] another of rather mixed type, and the tumor now under discussion is the seventh.

Briefly listing the articles dealing with this subject, there are two excellent ones by Ewing [17] and Vanzetti,[18] and other papers by Wiesel,[19] Barbano,[20] Roccavilla,[21] Simmonds,[22] Speed,[23] Perrero,[24] Letulle,[25] Chiari,[26] Paviot and Gerest,[27] Thiroloix and Debre,[28] Cleland and Beare,[29] Harvier,[30] Sweany[31] and Helvestine.[32] Some of these reports include more than one malignant tumor of the thymus.

Diagnosis of the Thymic Origin of these Tumors. It seems to be agreed that the following characteristics point to the thymic origin of a given tumor:

1. The growth is found in the anterior mediastinum at the site of the thymus and is firm and not very lobulated.
2. It does not tend to invade bone, extending down the sternum without involving it. The tumors reported by Symmers and Vance and by Jacobson both invaded vertebrae and constitute exceptions to this rule.
3. The pleura and pericardium are involved by direct extension, rather than by metastasis, and the lung may also be directly invaded.
4. There is a general resemblance of the type cells to those of the embryonic or post-embryonic thymus and the presence of structures resembling Hassal's corpuscles while helpful, is not essential to diagnosis.

5. The sarcomatous tumors occur in children or young adults, the carcinomatous in patients of "cancer age," in later life.
6. Metastasis below the diaphragm is very rare, being reported in only three cases: that of Ambrosini,[33] in Symmers and Vance's second case and in the case described by Friedlander and myself.

There is a good deal of dogmatic discussion in the earlier papers that is tending to give way to a more liberal interpretation of these tumors as our experience increases.

Special Consideration of this Case. The interesting features of the case we are discussing are: (*a*) It is definitely primary in the thymus and shows an unusually marked production of Hassal's bodies, not in the way Jacobson describes it in connection with his tumor, *i. e.*, by proliferation of "Hassal's corpuscle cells," but rather by a process of degeneration somewhat similar to that seen in the involuting thymus.* Ewing has already called attention to the similarity between this normal process and that seen in epithelial thymic tumors. (*b*) The invasion of the superior vena cava corresponds to the same feature noted by Letulle in his second case and, in a measure, to that in the inferior vena cava described in my third case (Friedlander and Foot[14]). This definitely indicates a transportation of tumor cells by the blood stream in the case of malignant thymic tumors and makes one wonder why metastases at a distance from these growths are so uncommon. (*c*) Another strange fact noted in this tumor is the comparative absence of true reticulum from its parenchyma, whereas reticulin fibers were abundant in the other tumors of my series, particularly the third. It was most abundant in the frank lymphosarcoma. If the thymic "reticulum cells" form the reticulum of the thymus, why should one find practically no reticulum in a tumor composed largely of cells that correspond very closely in their morphology with the embryonal "reticulum cells" and abundant reticulum in a growth whose type cell closely resembles the ordinary microlymphocyte? This point is worthy of further consideration.

* Since this paper was submitted for publication, H. L. Jaffé and Alexandra Plavska (*Proc. Soc. Exp. Biol. and Med.*, 1925, xxii, 91; and *Proc. Am. Soc. Exp. Path.* (to be published) have reported investigations on the transplanted thymus of the guinea-pig that tend to confirm the assumption that Hassal's corpuscles are degenerative products of the thymic reticuloid cells and are unconnected with the remnants of the epithelial ducts of Remak, or thymic vesicles.

Classification of Malignant Thymic Tumors in General. We are not interested here in the benign growths of this organ and shall consider only the malignant variety. The term "thymoma" applied to these tumors is significant. In naming a tumor one does not add "oma" to the stem designating an organ without implying a certain hesitancy as to the histogenesis of the growth; otherwise one would call it by a more specific name. This is particularly true in the case of thymic tumors. Because of the uncertainty that prevailed in connection with the histogenesis of the various cellular elements of the organ, one hesitated to call the tumors obviously originating in its cortex "lymphosarcomas" until the origin of the lymphoid cortical cell was established.

With three types of cell composing the organ one could, theoretically, expect tumors of epithelial, lymphoid and eosinophilic types, as well as a mixture of these. Several primary epithelial malignant growths have been described: typical adenocarcinomas; tumors resembling epidermoid carcinomas and containing more or less well developed Hassal's bodies, or "pearls"; and new growths in which the type cell approximates the entodermal thymic reticulum cell, with or without rudimentary Hassal's bodies. Carcinomas originating elsewhere and metastasizing to the thymus, or its rest, are not truly thymic and should be excluded from this category. The majority of malignant thymic tumors, however, have been of the general type of the lymphosarcoma with cells varying from large, round or ovoid bodies to small forms closely resembling microlymphocytes. The first and third tumors of my small series typify these two. The eosinophile type, which we have stated as an hypothetical possibility, has never been described so far as I know, and it does not seem likely that it ever will be if these cells be true blood eosinophiles. Ewing has noted the abundance of eosinophiles in certain thymic sarcomas and has drawn attention to the similarity of these to Hodgkin's granuloma. The fourth possibility, a tumor of mixed type, is already on record. Hare and Rolleston (Jacobson cit.) have described a dermoid cyst and a mixed tumor; and Harrington and I reported a malignant thymic tumor in which epithelial ducts and cysts alternated with large fields composed of thymic reticulum cells, interpreting the origin of both types as entodermal.

Let us consider the probable origin of the various types just enumerated. The sarcomatous is probably derived from the meso-

blastic elements that invade the thymus during its early development. The reason for this statement has already been sufficiently discussed. One must be on the alert, however, for types of thymic tumor that resemble the large spindle cell fibrosarcoma but are, in reality, made up of the entodermal reticulum cells and are therefore of epithelial nature. True fibrosarcoma might, indeed, arise from the fibrous perivascular tissue of the thymus; but we do not find it described thus far. Very often there will be a sufficient number of rudimentary Hassal's corpuscles to indicate entodermal origin in doubtful cases.

The epithelial tumors originating from entodermal tissue in the thymus are sufficiently definite in their type to render a diagnosis fairly simple. They may be composed of glandular complexes, alveoli or concentrically arranged masses of squamous cells identical in appearance with the typical thymic corpuscle. They probably originate from the entodermal vesicles of the organ and from their duct-like derivatives. Ewing has indicated that there may be some connection between tumors of this type and a disturbance in the normal thymic involution.

The resemblance between the mixed type of epithelial "thymoma" and some forms of mediastinal teratoma is striking and it may be that such a tumor as that described by Harrington and myself is, therefore, to be considered an embryoma, or unripe teratoma. For a discussion of the mediastinal teratomas the reader is referred to papers by Smith and Stone[34] and Böhmig.[35] Our tumor, however, could be sufficiently explained by a persistence of those epithelial elements which differentiate to form the thymic "reticulum" and the Hassal's corpuscles; cells originating in the primitive thymic entodermal vesicles and hence not totipotential (in the sense of the term as used by Adami) as are the parent cells of the teratoma and its malignant prototype, the embryoma. The tumor we described is apparently very uncommon, as none of the thymic tumors hitherto described exactly corresponds with it. It occurred in a young child and bears the same relation to the other thymic epithelial tumors that malignant epithelial growths in children bear to carcinomas in individuals of advanced age.

In closing, it is interesting to note the almost complete necrosis of the tumor reported in this article, a fact that is in all probability due to the prolonged treatment with deep radiotherapy. Groover,

Christie and Coe [36] have reported two cases in which thymic tumor was diagnosed clinically (but not proved pathologically) and in which deep radiation resulted in a marked remission in the tumor in one case and an apparently complete and permanent disappearance in another. Both of these occurred in adults, but it is impossible to tell what type of thymic tumor they represented.

A complete understanding of malignant thymic tumors will, of course, never be arrived at until we know more about the thymus and until the question of the etiology of malignant tumors in general has been answered. In the meantime, we may assist investigators of both these questions by carefully studying and reporting every case of "malignant thymoma"; for the study of teratology has already accumulated much evidence which is of distinct value to the embryologist and histologist, as well as to the science of medicine.

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DESCRIPTION OF PLATES

PLATES 11–14

FIG. 1. A portion of the mediastinal tumor showing necrosis at bottom and a large Hassal's corpuscle at top; separating them is a mass of loose connective tissue. X 500.

FIG. 2. Metastasis to the lung, tumor growing in the air sacs. A mass of necrotic tissue with two Hassal's bodies (or two portions of a partially necrotic one) is seen in one corner. X 500.

FIG. 3. Metastasis to a lymph node; note the duct-like grouping of some of the cells, the hyalinized masses of concentrically arranged cells and the loose reticulum of tumor cells. X 500.

FIG. 4. Metastasis to the lung showing that the only true reticulum is that of the pulmonary alveolar framework. The very fine threads connecting various tumor cells are cytoplasmic processes, not true fibers. X 500.

FIG. 5. The beginnings of a Hassal's corpuscle; two hyalinized cells at the center of the picture represent this stage. X 1,000.

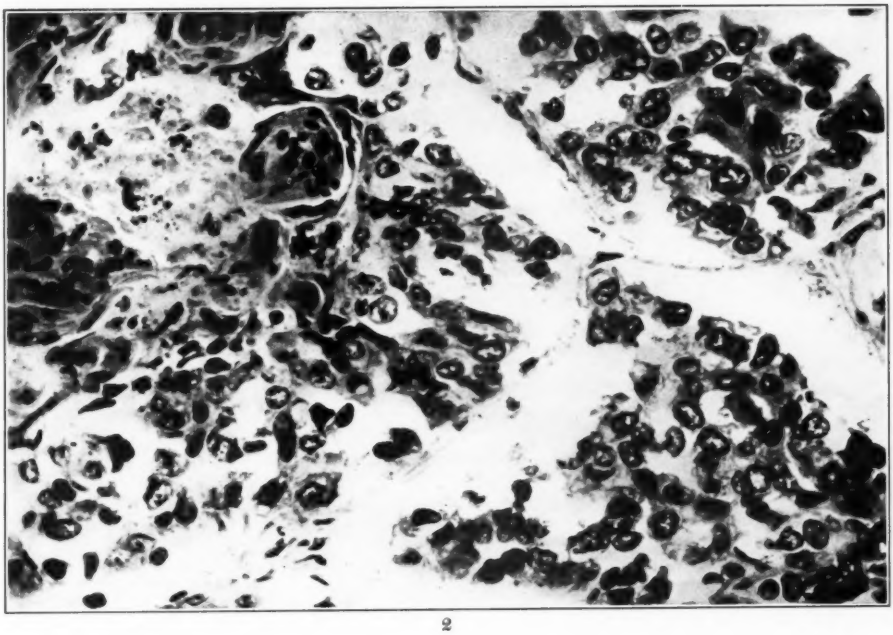
FIG. 6. Somewhat larger group of hyalinized cells, the nuclei becoming pyknotic. Probably intermediate between the preceding and following pictures. X 1,000.

FIG. 7. Portions of two completed Hassal's corpuscles with beginning hyalinization, or at least much increased density, of the cytoplasm of a large field of tumor cells. Other tumor cells retain their faint, spidery outlines. X 1,000.

All the sections from which these photomicrographs were taken were fixed in Zenker's fluid, cut at five microns and stained with Harris' hematoxylin and eosin, except that for Fig. 4 which was impregnated with silver and counterstained with the Van Gieson method to demonstrate reticulum.

1

Foot Malignant Thymoma



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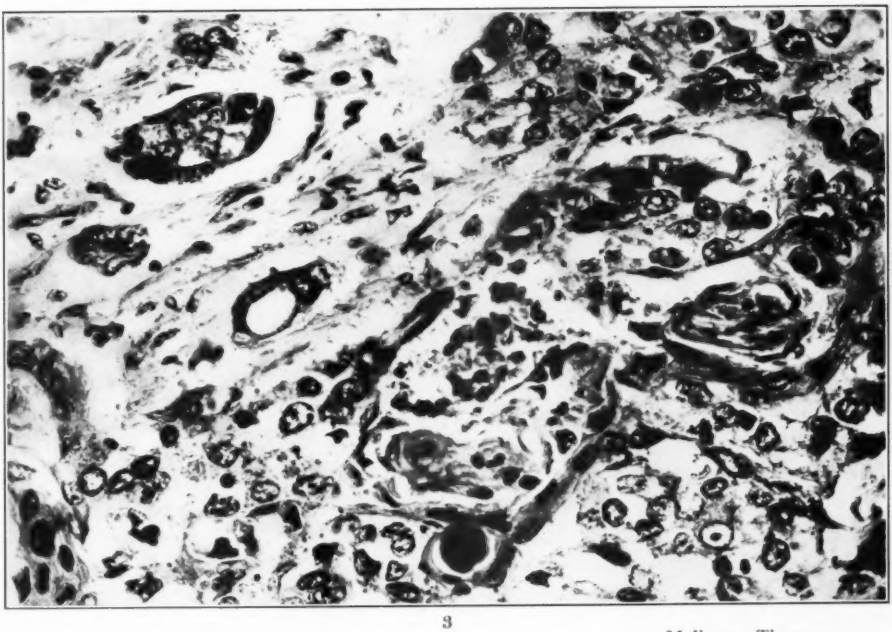
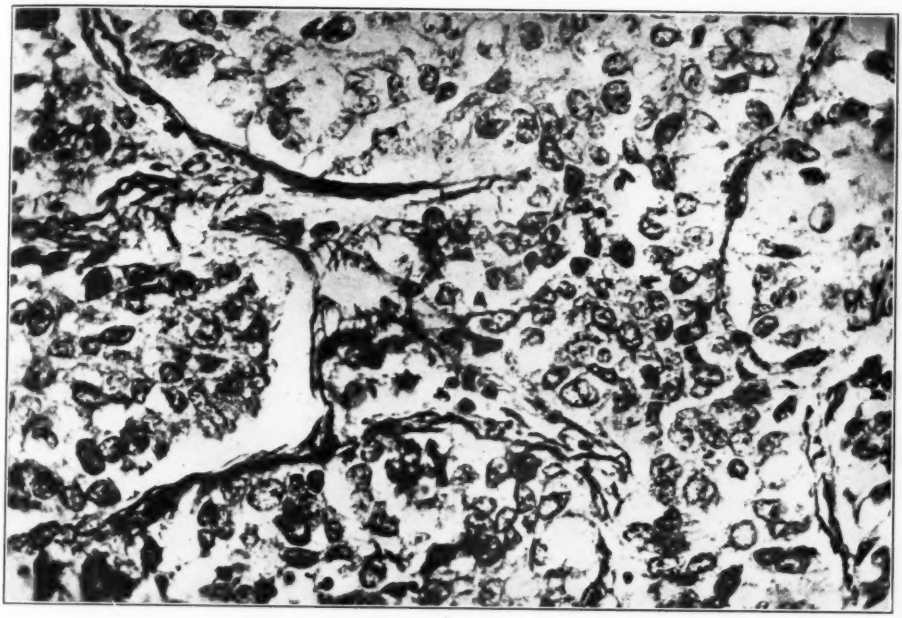


3

Foot

Malignant Thymoma



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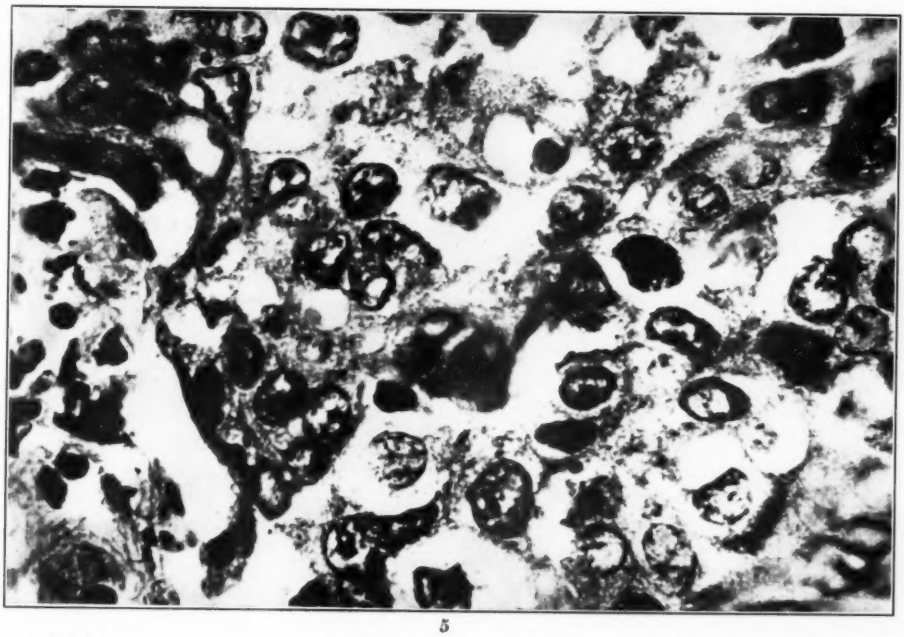
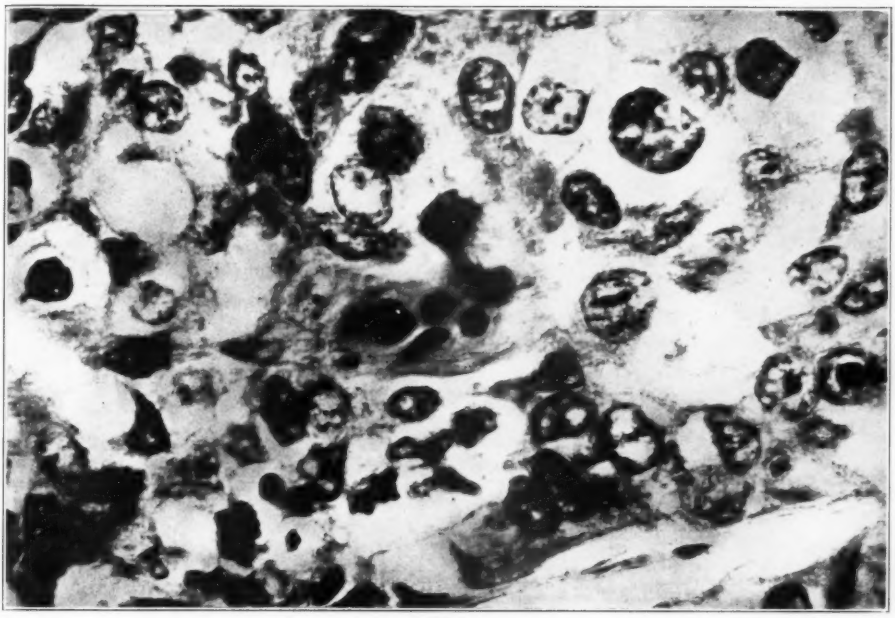


5

Foot Malignant Thymoma



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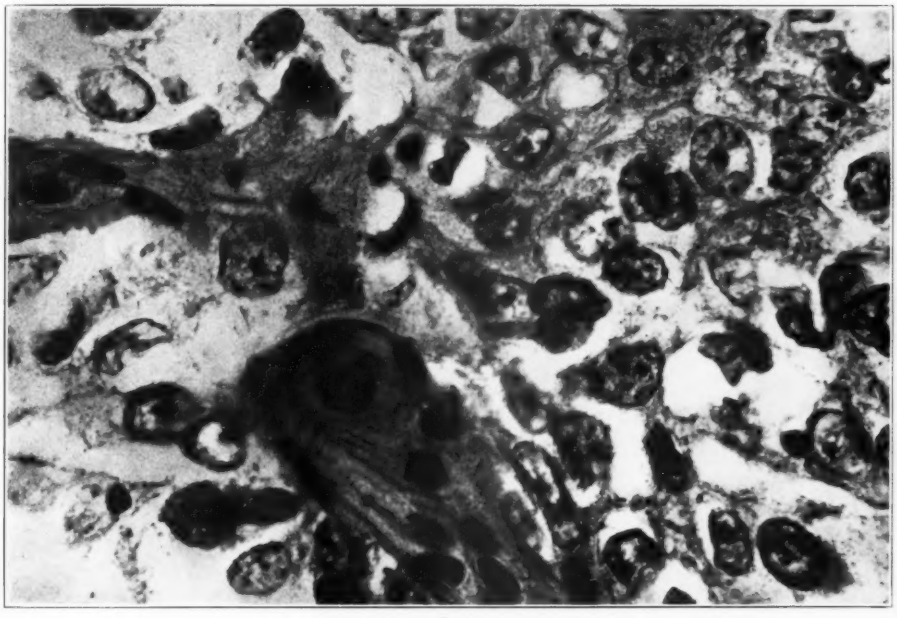

7

Foot Malignant Thymoma

THE FATE OF REACTING LEUCOCYTES IN THE TUBERCULIN AND REINFECTION REACTIONS *

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INTRODUCTION

In two previous studies [1, 2] concerned with the tuberculin reaction and the reaction to tuberculous reinfection in testes of hypersensitive guinea-pigs, one of the authors noted a rather marked phagocytosis of reacting polymorphonuclear leucocytes by endothelial leucocytes. The question naturally arose as to whether or not these phagocytized polymorphonuclears were dead before they became engulfed. It is believed that solution of this question might have a certain theoretical value in interpreting the protective significance of the tuberculin and reinfection reactions.

Nearly all laboratory workers in tuberculosis believe that when an animal is tuberculin hypersensitive, there is a certain variable degree of immunity toward reinfection with the tubercle bacillus. It is likewise quite tacitly assumed that the two phenomena are causally related; that is to say, the more immediate inflammatory reaction to reinfection in the hypersensitive animal is responsible for the slower dissemination of bacilli, which is known to occur.[3] There are, however, certain quite apparent objections to this ready assumption. In the tuberculin and in the reinfection reaction the leucocytes forming the bulk of the exudate are polymorphonuclear; so far as is known, the polymorphonuclear leucocyte phagocytizes tubercle bacilli but does not destroy them; consequently the rôle played by these cells in the reaction is problematical. The mononuclear leucocyte is believed by some to destroy tubercle bacilli and Krause and Peters [4] observed in the reinfection reaction an earlier appearance of mononuclears than in non-sensitized control animals

* Received for publication November 4, 1925.

with primary infection. To them this observation suggests an explanation of the protective value of sensitization. Recently Cunningham, Sabin, Sugiyama and Kindwall [5] have denied that the monocyte destroys tubercle bacilli. We do not know the reason for the polymorphonuclear or for the mononuclear reaction in reinfection. Our own observations in the past have led us to favor its interpretation as a response to local tissue injury. Whether this is the sole explanation or whether some further mechanism comes into play, cannot be determined on the basis of present knowledge. It has seemed to us desirable to know if the leucocytes themselves share in the general sensitization of the tuberculous animal and the present study is concerned with this point.

Methods

One method of demonstrating sensitization is to show whether or not the supposedly sensitized cell undergoes necrosis when exposed to specific agents, in this case tuberculin, which to the cells of normal animals are innocuous. We first attempted to obtain a non-specific exudate in an animal sensitized by killed tubercle bacilli by the use of aleuronat and to incubate this exudate with varying amounts of old tuberculin, determining the death of the leucocytes by Nakayama's method.[6] The method is cumbersome, ill-suited to small amounts of material and uncertain; after a few trials it was abandoned. It was next decided to attempt the reaction *in vivo*, withdrawing the exudate from time to time and determining the condition of the cells by supravital staining with neutral red and Janus green following the technic of Sabin and her collaborators.[7, 8]

The important point in the supravital technic is to have a clean glass slide evenly covered with the vital dye. Glass slides are placed in a solution of concentrated sulphuric acid and potassium bichromate for three days. The slides are then washed in tap water for one day, rinsed thoroughly with distilled water and stored in 80 per cent alcohol. Saturated alcoholic stock solutions of Janus green and neutral red are prepared. A dilute solution of neutral red is then made by adding twenty drops of the stock solution to 5 cc. of absolute alcohol. Three drops of the saturated Janus green solution are added to 2 cc. of the dilute neutral red solution. This gives the dilution of the combined dye necessary for the differential

staining of the supravital technic. The glass slides are then removed from the 80 per cent alcohol by means of forceps, dried with a clean piece of gauze, flamed and then flooded with the combined dye which is quickly drained off so as to obtain an equal distribution on drying. It is essential that the fingers touch neither the slide nor the dye lest there be contamination with small amounts of grease. The stained slides are then stored in a slide box until they are used.

A series of guinea-pigs were sensitized by the injection of killed tubercle bacilli into the peritoneal cavity. Three injections, each 2.5 mg. dry weight of bacilli, sufficed. Ten days after the last treatment all of the animals reacted strongly to an intracutaneous inoculation of old tuberculin in 1:20 dilution. With one exception, the skin tests did not give necrosis, showing only marked erythema, induration and central blanching. Several days elapsed between the skin testing and the examination of the exudates. The latter were obtained as follows: two guinea-pigs, one sensitized and one control, each received 4 cc. of hormone broth containing 0.1 cc. of old tuberculin into the right pleural cavity; no immediate reaction resulted, save for a temporary series of hiccoughs. Twenty-four hours later the exudates were withdrawn, using glass tuberculin syringes previously subjected to the cleansing treatment described for slides covered with the stain. Syringes were warmed to body temperature before use. Needles were cleaned in alcohol followed by ether. A drop of the exudate was placed on a cover slip which was quickly inverted on the warm slide; the preparation was ringed with vaseline of high melting point and transferred to a microscope in a warm box maintained at a temperature of 37 to 38 C. Fluids from control and sensitized animals were placed on the same slide to avoid errors due to concentration of dye or other possible difficulties. This procedure was repeated at forty-eight hours and in some instances again at seventy-two hours.

At this point it becomes necessary to discuss the criteria for identifying the living, injured and dead cells observed in the supravital preparations of the exudate (Figs. 1 to 8). The polymorphonuclear leucocyte is characterized in the supravital preparation by its motility. These cells in their most active state show a streaming of the neutrophilic granules with the nucleus following behind. In the resting state these cells are round and show slight streaming and Brownian movement of the neutrophilic granules. The monocyte

shows motility, but to a less degree than the polymorphonuclear. In these cells there is a clear centrosphere in the cytoplasm occupying the indented portion of the nucleus surrounded by a rosette of small pink-staining bodies. Outside the rosette are the larger vacuoles staining intensely with neutral red. The mitochondria staining a deep bluish green with the Janus green are always on the periphery of the vacuoles. In the monocyte phagocytized material is always placed peripherally to the rosette of small bodies and the centrosphere is never obscured. The monocytes become round in the inactive stage. The clasmatocytes are large cells showing very little motility and a remarkable phagocytic ability. No centrosphere or rosette is visible and the mitochondria are not as prominent as in the monocyte. The phagocytized material is distributed throughout the cytoplasm. The material first phagocytized is placed close to the nucleus and the cell fills up with débris irregularly and according to no definite pattern. These cells exhibit vacuoles of different sizes staining in varying degree with neutral red. The lymphocytes are characterized by very slow ameboid movement, the nucleus preceding the cytoplasm. In the inactive state they are round and show very little cytoplasm. There are numerous rod-shaped or dot-like mitochondria and very often one or two small neutral red staining bodies localized in one part of the narrow rim of cytoplasm. In the supravital preparation, cell injury is denoted by rounding of the cells, together with cessation of the streaming motions of the granules. Then as the cell dies there is a gradual diffusion of the neutral red throughout the protoplasm of the cell together with a concentration of the granules, vacuoles, mitochondria and nuclear material in the center of the cell. The nucleus is deeply stained, whereas in the living cell it is unstained.

Protocols

Guinea-pig 244 (sensitized) received 4 cc. hormone broth with 0.1 cc. O. T. in right pleural cavity. Twenty-four hours later the exudate contained:

dead polymorphonuclears, 81 per cent; living, none;
dead lymphocytes, 1 per cent; living, none;
dead monocytes, 18 per cent; living, none.

No further examinations were made.

The corresponding control guinea-pig received identical treatment. The pleural fluid showed:

living polymorphonuclears, 26 per cent; dead, none;
living eosinophiles, 14 per cent; dead, none;
living monocytes, 57 per cent; dead, none;
living clasmatocytes, 3 per cent; dead, none.

No subsequent examination was made.

GUINEA-PIG 242 (sensitized) received broth and tuberculin as did the above. Twenty-four hours later the exudate consisted of:

dead polymorphonuclears, 92 per cent; living, none;
dead monocytes, 8 per cent; living, none.

At 72 hours:

living polymorphonuclears, 21 per cent; dead, none;
living basophiles, 1 per cent; dead, none;
living eosinophiles, 4 per cent; dead, none;
living small lymphocytes, 21 per cent; dead, none;
living monocytes, 40 per cent; dead, none;
living clasmatocytes, 13 per cent; dead, none;
living clasmatocytes, 8 per cent; dead, none.

The control animal showed at 24 hours:

living polymorphonuclears, 79 per cent; dead, none;
living eosinophiles, 4 per cent; dead, none;
living small lymphocytes, 1 per cent; dead, none;
living monocytes, 16 per cent; dead, none.

At 72 hours:

living polymorphonuclears, 29 per cent; dead, none;
living basophiles, 3 per cent; dead, none;
living eosinophiles, 1 per cent; dead, none;
living small lymphocytes, 5 per cent; dead, none;
living monocytes, 54 per cent; dead, none;
living clasmatocytes, 8 per cent; dead, none.

GUINEA-PIG 245 (sensitized) was treated as above. In 24 hours the exudate showed:

dead polymorphonuclears, 92 per cent; living, none;
dead small lymphocytes, 2 per cent; living, none;
dead monocytes, 6 per cent; living, none.

At 48 hours:

living polymorphonuclears, 17 per cent; dead, 14 per cent;
living lymphocytes, 12 per cent; dead, none;
living monocytes, 56 per cent; dead, none;
living clasmatocytes, 1 per cent; dead, none.

At 72 hours:

living polymorphonuclears, 5 per cent; dead, none;
living lymphocytes, 20 per cent; dead, none;
living monocytes, 61 per cent; dead, none;
living clasmatocytes, 14 per cent; dead, none.

The corresponding control gave at 24 hours:

living polymorphonuclears, 88 per cent; dead, none;
living eosinphiles, 3 per cent; dead, none;
living lymphocytes, 1 per cent; dead, none;
living mononuclears, 8 per cent; dead, none.

At 48 hours:

living polymorphonuclears, 65 per cent; dead, none;
living eosinophiles, 1 per cent; dead, none;
living lymphocytes, 1 per cent; dead, none;
living monocytes, 31 per cent; dead, none;
living clasmatocytes, 1 per cent; dead, none;
living serosal cells, 1 per cent; dead, none.

At 72 hours:

living polymorphonuclears, 28 per cent; dead, none;
living lymphocytes, 6 per cent; dead, none;
living monocytes, 60 per cent; dead, none;
living serosal cells, 6 per cent; dead, none.

GUINEA-PIG 241 (sensitized). Identical treatment was given as above. At 24 hours:

dead polymorphonuclears, 95 per cent; living, none;
dead lymphocytes, 5 per cent; living, none.

At 48 hours:

living polymorphonuclears, 33 per cent; dead, 48 per cent;
living monocytes, 18 per cent; dead, none;
living clasmatocytes, 1 per cent; dead, none.

At 72 hours:

living polymorphonuclears, 49 per cent; dead, none;
living eosinophiles, 9 per cent; dead, none;
living lymphocytes, 11 per cent; dead, none;
living monocytes, 28 per cent; dead, none;
living clasmatocytes, 3 per cent; dead, none.

The control guinea-pig showed at 24 hours:

living polymorphonuclears, 87 per cent; dead, none;
living monocytes, 12 per cent; dead, none;
living clasmatocytes, 1 per cent; dead, none.

At 48 hours:

living polymorphonuclears, 67 per cent; dead, 1 per cent;
living eosinophiles and basophiles, 1 per cent each; dead, none;
living monocytes, 27 per cent; dead, none;
living clasmatocytes, 3 per cent; dead, none.

At 72 hours:

living polymorphonuclears, 61 per cent; dead, none;
living eosinophiles, 3 per cent; dead, none;
living monocytes, 36 per cent; dead, none.

GUINEA-PIG 239 (sensitized). This animal, instead of receiving tuberculin, was given a suspension of killed tubercle bacilli made by adding 1 cc. of saline suspension of bacilli, containing 2.5 mg. per cc., to 2 cc. of hormone broth. At 24 hours the exudate contained:

living polymorphonuclears, 53 per cent; dead, 20 per cent;
living eosinophiles, 7 per cent; dead, none;
living monocytes, 20 per cent; dead, none.

At 48 hours:

dead polymorphonuclears, 89 per cent; living, none;
dead lymphocytes, 9 per cent; living, none;
dead monocytes, 1 per cent; living, none;
dead clasmatocytes, 1 per cent; living, none.

At 72 hours:

living polymorphonuclears, 38 per cent; dead, 2 per cent;
living eosinophiles, 4 per cent; dead, none;
living lymphocytes, 16 per cent; dead, none;
living monocytes, 30 per cent; dead, none;
living clasmatocytes, 10 per cent; dead, none.

The control under similar treatment showed at 24 hours:

living polymorphonuclears, 81 per cent; dead, none;
living eosinophiles, 4 per cent; dead, none;
living monocytes, 15 per cent; dead, none.

At 48 hours:

living polymorphonuclears, 55 per cent; dead, none;
living lymphocytes, 2 per cent; dead, none;
living monocytes, 37 per cent; dead, none;
living clasmatocytes, 6 per cent; dead, none.

At 72 hours no fluid could be obtained.

It is interesting to note the variation in phagocytosis occurring from day to day in both sensitized and control animals. At the end of twenty-four hours there were very few monocytes and practically no clasmatocytes present in the exudate and very little phagocytosis by these cells. After forty-eight hours the exudate showed numerous phagocytic cells, chiefly monocytes, containing débris and whole polymorphonuclears; after seventy-two hours the monocytes and clasmatocytes were again increased in numbers and in phagocytic activity. It was not uncommon to see a clasmatocyte containing from three to seven polymorphonuclears and monocytes full of cellular débris. Phagocytosis was more marked always in the later stages of the reaction in the sensitized animal than in the control.

Discussion

The results derived from this series of experiments are difficult to interpret in immunologic terms. It is certain that evidence points to the fact that in the tuberculin and reinfection reactions the reacting cells are sensitized and in early stages of the reaction undergo necrosis. With a diminution of the intensity of the reaction due possibly to the absorption of the exciting antigen, barely possibly to a desensitization, new polymorphonuclears appear as a reaction to tissue débris and with them come large numbers of mononuclear phagocytes. The whole phenomenon suggests that the leucocytes appearing in the tuberculin reaction do not have the protective significance commonly ascribed to them. To determine this point a huge series of animals would be necessary, with varying reinfecting doses of living organisms and correlation of spread of disease with the results of studying the behaviour of cells in the immediate reaction. One other interpretation is possible: the observed diminished rate of spread of reinfecting organisms in a hypersensitive animal may be due to necrosis and disintegration of the cell carrying them, this necrosis occurring near the source of inoculation rather than at a distant focus. No positive conclusion as to the immunologic significance of facts so far observed is justified.

Conclusions

In the tuberculin and reinfection reactions in tuberculin hypersensitive animals, the reacting inflammatory cells appear to be themselves sensitized. Treated with appropriate doses of antigen, they undergo early death.

These first cells are gradually replaced by others probably reacting largely to the presence of necrotic tissue. The newly emigrating cells are viable elements.

Further study is required to determine the immunologic significance of these facts.

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DESCRIPTION OF PLATE

PLATE 15

FIG. 1. Dead polymorphonuclear neutrophile; nucleus deeply stained, cytoplasm diffusely stained with the granules clumped about the nucleus leaving a peripheral clear zone.

FIG. 2. Dead monocyte; a narrow peripheral clear zone within which is diffusely stained cytoplasm and many neutral red bodies. At the upper right a dead phagocytized polymorphonuclear; beneath this the faint, diffusely stained nucleus of the monocyte.

FIG. 3. Motile, active polymorphonuclear neutrophile; nucleus unstained; granules occasionally stained; several pseudopods.

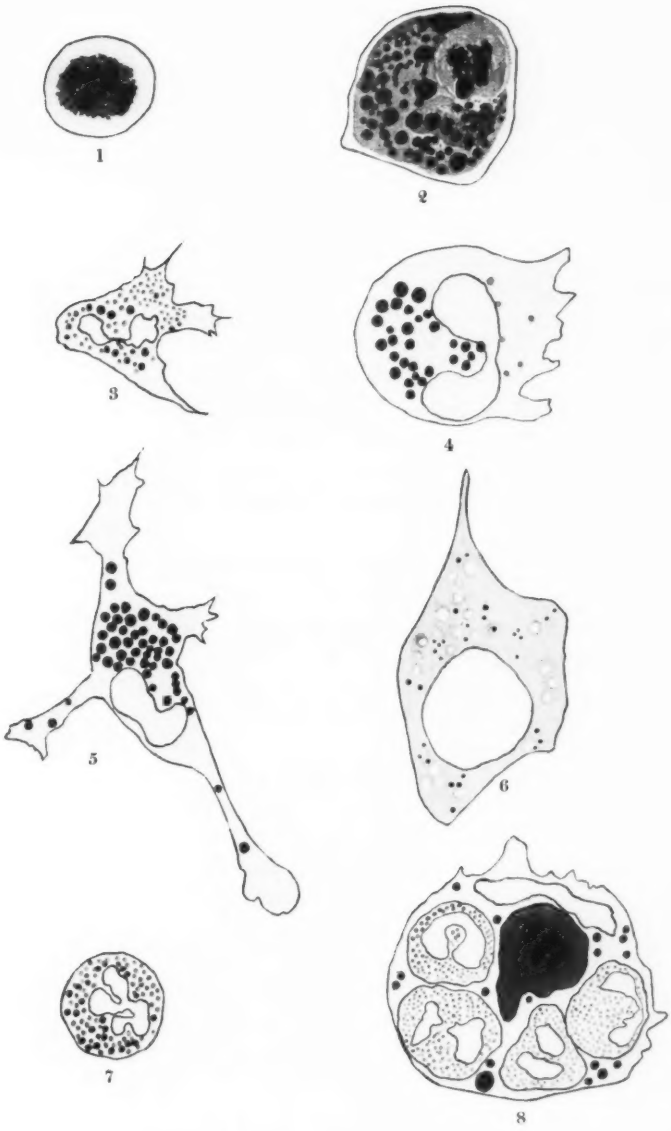
FIG. 4. Resting monocyte; crescentic unstained nucleus; neutral red staining material occupying the cytoplasm adjacent to the hollow crescentic portion of nucleus; a few small pseudopods.

FIG. 5. Very active monocyte; numerous long pseudopods.

FIG. 6. Living serosal cell; nucleus unstained; no neutral red staining material.

FIG. 7. Resting polymorphonuclear neutrophile. The cell is rounded; granules fill out the cytoplasm; nucleus unstained.

FIG. 8. Living clasmatocyte. Flattened eccentric nucleus, unstained; neutral red staining material scattered sparsely throughout cytoplasm; five phagocytized polymorphonuclears, four of which are living and one (stained) dead.



Stewart, Long and Bradley

Fate of Leucocytes in Tuberculin Reaction

THE PERSISTENCE OF THE GLOMERULAR CIRCULATION FOLLOWING OCCLUSION OF THE RENAL VEIN OF ONE KIDNEY IN THE CAT*

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Only the immediate pathologic changes consequent to thrombosis of the renal vein in man are well known, but possibly certain lesions found in the kidney are the late results of previous venous occlusions which have been survived, and the changes are not recognized as lesions resulting from this occurrence. With a view of determining experimentally whether any characteristic changes followed venous obstruction of the kidney, the left renal vein in a series of cats was cut between ligatures, and the animals killed at varying intervals of time thereafter. The resulting changes seemed to furnish data on the finer circulation of the kidney not furnished by other experimental methods.

In brief, the distinctive feature of the picture following venous obstruction is the preservation of glomeruli in contrast to the widespread destruction of tubules. The early changes consisted of congestion and edema followed by degeneration and necrosis of convoluted tubules, most marked in the peripheral zone of the cortex. Beneath this peripheral zone is another zone of less marked degeneration of tubules where glomeruli, though showing changes in the capsular epithelium, were relatively undamaged. At a later period the glomeruli presented a healthy appearance, while there was complete destruction of tubules. At the end of the second month there was an extremely atrophied organ, reduced to about one-twentieth the volume of the opposite kidney, and consisting almost wholly of intact, closely approximated glomeruli.

Injections of Berlin blue through the renal artery of these atrophied kidneys showed a free circulation through the glomeruli. The path of this circulation could be traced through the kidney in microscopic sections of the injected organ, from the main renal artery and its larger branches, through afferent arterioles to the glomerulus; and

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thence through postglomerular vessels to extrarenal anastomosing channels. That the anastomosing circulation occurred through already existing channels was indicated by the uniformity of the results and the similarity of the collateral circulation traced in the case of normal and atrophied kidneys. It was clearly established that this collateral circulation involved only the glomeruli and excluded the tubules, both convoluted and collecting. In other words, the vessels to the tubules alone are terminal vessels, and this is responsible for their complete destruction following vascular stasis. In contrast to the blood supply to the tubules, the circulation through the glomeruli was found to be an extremely free one, as the injection with Berlin blue was accomplished more readily and with less pressure than in the case of the normal kidneys. When adhesions occurred between the omentum and the injured kidney, injections of blue showed that no anastomosing channels were established through these points of adhesions.

There is then a certain independence of the vascular supply of the glomeruli from that of the tubules and this can be conceived of as having some bearing upon the functional independence of these two elements.

Experimental Procedure

Young adult cats were anesthetized and the left renal vein cut between ligatures. In several instances the spermatic or ovarian veins were also sectioned. At varying intervals of time up to four months, the animals were killed. In all cases, the vessels were carefully dissected after death to study the collateral circulation and to prove that the renal artery was patent and that the renal vein had been cut proximal to its tributaries.

In several instances while the kidneys lay *in situ*, the renal arteries were injected with a 2 per cent solution of Berlin blue and the course of the injected vessels studied in thick microscopic sections with a binocular microscope.

The operation was performed on fourteen cats and one dog. Typical experiments have been selected from the series and are described below in the order of the time interval following ligation.

Experiment 1. Cat 17. Thirty-two hours after ligation.

Grossly, the kidney is swollen, tense and purple. Blood has extravasated into the retroperitoneal tissues. The capsule strips

readily, disclosing mottled petechial hemorrhages on the surface of the kidney. On section, the medulla is dark purple and is clearly demarcated from the pale opaque swollen cortex in which all markings are obscured.

Microscopically, there is complete necrosis of certain segments of the tubules, while other segments are well preserved and the glomeruli remain intact. The distribution of necrosis is so regular that a definite pattern is formed. The periphery of the cortex immediately beneath the capsule contains nothing but tubules, cells of which have lost all nuclei; the cytoplasm stains deeply with eosin and fills up the entire lumen. The basement membrane remains intact, and sharply defines the outer border of each degenerated tubule. From this outer necrotic zone the loops of Henle extend inward as wedge-shaped areas of degenerated tubules. Collecting tubules traversing the pyramid are similarly necrotic. In the zone between the wedge-shaped areas of necrotic tubules, relatively intact, proximal convoluted tubules surround each glomerulus. Their cuboidal cells contain clearly stained nuclei and have only slightly vacuolated cytoplasm. The glomeruli are without lesions. Their capillaries appear patent and their capsules are not thickened. The subcapsular space is clear. All glomeruli appear much the same. There is none showing any stage of fibrosis or atrophy. The medulla contains only necrotic tubules in which the basement membrane remains intact, and many engorged capillaries. There is no cellular reaction around the necrotic tubules. The main vein is occluded by thrombosis.

EXPERIMENT 2. Cat 19. Eight days after ligation.

Grossly, the left kidney is of about the same size as the right. The capsule is slightly adherent, due to organization of mottled areas of subcapsular hemorrhage. On section the cortex presents a peripheral rim of bright yellow sharply defined by a narrow hemorrhagic zone from the dull grayish yellow appearance of the inner portion of the cortex. A hemorrhagic zone separates the inner border of the cortex from the medulla.

Microscopically, necrosis of tubules has occurred according to a definite pattern. In the periphery of the cortex there is complete necrosis of tubules and of the few glomeruli that are in this area (Fig. 1). This zone is clearly defined by its intense staining of the necrotic protoplasm with eosin. All nuclei have disappeared from

the epithelial cells. The outlines of tubules, however, are indicated by the intact basement membrane and by the fact that fibroblasts are proliferating in the interstices between preexisting tubules. This proliferation of fibroblasts is from the surface of the kidney and the cells are penetrating inward. The necrotic tubules extend from the periphery downward, forming triangular areas with the apices pointing inward and reaching the medulla, and representing the loops of Henle and collecting tubules.

In the deeper zone of the cortex between the descending columns of necrotic tubules, are tubules that show some regeneration of the epithelial cells. Their cellular appearance contrasts markedly with the necrotic tubules. Their nuclei are large and closely placed, the cells being heaped upon the basement membrane in an irregular manner without definite alignment. Other tubules in this zone contain casts of hyaline material.

In the medulla the tubules are necrotic, only the basement membrane indicating preëxisting tubules. Capillaries here are patent and engorged. Near the pelvis and near the cortico-medullary junction fibroblasts are proliferating.

Glomeruli throughout, except in the peripheral zone, are well preserved. Their capillaries contain red cells. The subcapsular space is of usual size and no thickening of the capsule has occurred. The glomeruli are closely placed due to destruction of intervening tubules.

EXPERIMENT 3. Cat 30. Seventeen days after ligation.

In this case part of the collateral circulation had been cut off by ligating the ovarian vein as well as the renal vein.

Grossly, the omentum, spleen and pancreas are adherent to the left kidney. There are distended vessels in the lateral abdominal wall near the kidney. Blood has extravasated retroperitoneally. The left kidney is atrophied to about one-half the size of the right.

While the kidneys lay still ***in situ***, the renal arteries of both sides were injected with 2 per cent Berlin blue. The solution passed into the left kidney more readily than into the normal one. The dye quickly filled the collateral circulation consisting chiefly of branches to the adrenal, to the lateral abdominal wall and lumbar region. The same collateral channels were injected on the normal side by injecting the right renal artery. No vessels were stained in the adhesions formed between omentum, pancreas and kidney.

The capsule of the left kidney can not be stripped away. On section, the deeper portion of the cortex is stippled by dark blue dots representing injected glomeruli which are more closely placed than in the normal kidney. There is a pale peripheral zone of the cortex which contains little blue pigment, while in the healthy kidney the pigment reaches the outermost portion of the cortex. A few blue strands traverse the pale medulla and reach the cortex and surface of the kidney. The lining of the pelvis stains a deep blue.

Microscopically, old hemorrhage beyond the capsule is in the process of organization and fibroblasts extend from without into the renal tissue. The cortex shows a peripheral zone of necrosis where tubules are defined only as masses of hyalinized protoplasm bordered by basement membrane and separated by fibroblasts.

Beneath this outer zone, the cortex contains tubules in various stages of degeneration and atrophy. Some tubules show regenerating cells which are atypical in arrangement, the cells being heaped up without definite alignment. The nuclei stain well and the cells are so numerous that the nuclei appear closely placed. These cells practically fill the lumina. The impression given is that the cells have regenerated due to connection with the glomerular circulation but that they are relatively undifferentiated in character and nonfunctioning. In some tubules the lumen is dilated and contains granular material or hyaline casts. Among these atypical tubules the glomerular tufts stand out prominently. Their capillaries are well filled with deep blue pigment. The capsules are not thickened and the subcapsular space is of usual size and empty.

In the medulla, the central portion is completely necrotic, only shadows of tubules remaining, while the pelvic border and corticomedullary junction show proliferating fibroblasts and capillaries.

Throughout the sections, vessels filled with blue pigment are seen: (1) large arterial branches at the hilum, (2) arteries in cross section at the cortico-medullary junction, (3) afferent arterioles leading to the glomeruli, (4) longitudinal sections of postglomerular vessels running through all zones of the cortex, including the necrotic peripheral zone, and reaching the surface, (5) capillaries in the perirenal area of organizing hemorrhage and (6) capillaries in the medulla just beneath the pelvic lining. Thus the circulation can be traced from the artery at the hilum through perforating vessels which bear glomeruli, to the perirenal vessels.

In the normal right kidney the capillaries injected with blue are more numerous and are seen to pass to healthy tubules as well as to glomeruli. The injected glomeruli are identical in appearance to those of the left kidney.

EXPERIMENT 4. Cat 31. Five weeks after ligation.

The left ovarian vein had been ligated, as well as the renal vein. Adhesions have formed between the omentum and colon and the left kidney. While the kidneys lay *in situ*, the renal arteries were injected with Berlin blue. The collateral circulation thus injected was similar to that previously described. The adherent omentum and intestine were not stained by this injection, and consequently played no part in the collateral circulation.

There is some hemorrhage in the retroperitoneal tissues. The capsule is so adherent that it is impossible to strip it from the kidney. The kidney is smaller than the right. On section, the medulla is unstained except near the pelvis and at the cortico-medullary junction. The peripheral layer of the cortex is bright yellow, necrotic and contains no blue. The inner portion of the cortex is stippled by blue points representing injected glomeruli. In the normal kidney the medulla is deeply stained with blue and the entire cortex contains blue dots and striations.

Microscopically, the peripheral portion of the cortex contains no healthy tubules. A growth of fibroblasts extends inward from the capsule and penetrates in strands between the remains of tubules. Only the basement membranes of tubules remain and appear as small cystic spaces riddling the growth of connective tissue. In this zone no glomeruli are present. Large capillaries filled with blue extend through this zone to the surface.

The cortex beneath this outer zone contains healthy glomeruli well filled with blue (Fig. 2). An occasional glomerulus is atrophied. Tubules in this area are largely atrophied or show lumina filled with hyaline casts. The medulla is completely necrotic and contains no vessels filled with blue, except the portion immediately contiguous to the pelvis and to the cortex. The capillary circulation is quite similar to that previously described.

EXPERIMENT 5. Cat 20. Two months after ligation.

Grossly, the left kidney is reduced to an extremely small organ, measuring 1.8 × 1.0 × 0.6 cm., and weighing 2.1 gm., while the

right kidney measures 4.5 × 3.2 × 2.5 cm., and weighs 25.1 gm. The capsule strips readily. On section, the tissue is an orange-yellow color. The cortex is very narrow. The renal artery is patent.

Microscopically, a sharply defined peripheral zone of the cortex contains atrophied or hyalinized tubules and a diffuse growth of fibroblasts. Immediately beneath this zone, the cortex consists of many glomeruli, closely approximated because of atrophy of tubules (Fig. 3). A few extremely atypical tubules survive. The glomeruli appear patent, and the subcapsular space is of usual size. In the medulla, empty spaces apparently represent atrophied tubules. These are supported by connective tissue and capillaries.

EXPERIMENT 6. Cat 33. Two months after ligating renal vein.

The renal arteries of both sides were injected with Berlin blue while the organs lay *in situ*. The usual collateral circulation was thereby demonstrated. The left kidney is enormously atrophied measuring 1.5 × 1.3 × 1.0 cm., while the unligated right kidney measures 4.8 × 3.5 × 2.5 cm. The atrophied kidney shows only a narrow rim of uninjected cortex. The medulla is pale.

Microscopically, all tubules have atrophied or have disappeared, with only the supporting connective tissue remaining. Many glomeruli have likewise been destroyed, but those which persist have been permeable to the injected Berlin blue which is seen filling their capillaries. The glomeruli are closely packed due to atrophy of intervening tubules. Their capsules are thickened and the subcapsular space is enlarged.

EXPERIMENT 7. Dog 5. Three months after ligation.

Grossly, the left kidney is reduced to a minute organ measuring 2.8 × 1.9 × 1.2 cm., while the right measures 6.8 × 3.5 × 3.3 cm. The capsule strips readily. On section the tissue is of an orange-yellow color. The medulla is reduced to a narrow yellow zone. The renal artery is patent.

Microscopically, there is a clearly defined peripheral zone of the cortex in which tubules are completely atrophied and replaced by connective tissue, but in which the capillaries are patent. The entire cortex below this is composed of glomeruli, closely approximated because of atrophy of intervening tubules (Fig. 4). Some atypical tubules persist. Everywhere the capillaries are apparently patent.

The medulla consists of a diffuse growth of connective tissue and atrophied collecting tubules indicated chiefly by basement membrane.

In the case of three of the cats, in which the ovarian or spermatic veins had been ligated in addition to the renal vein, no collateral circulation was functioning and there was consequent complete necrosis of all elements. In other animals in which the ovarian vein had been ligated, as well as the renal, the changes were the same as when the renal vein alone had been ligated.

Discussion

In reviewing the literature, it was found that the changes within the kidney resulting from experimental venous occlusion had been described in 1876 by Buchwald and Litten [1] who attempted in this manner to reproduce the contracted kidney in rabbits and dogs. They describe especially the early stages of congestion and edema followed by destruction of tubules. They state that in contrast to the universal destruction of tubules, the preservation of the glomeruli is striking. The organ decreased markedly in size and the collateral circulation was abundant.

The circulation of the kidney has been studied usually with the object of determining the arterial distribution under normal and pathologic conditions by various injection and corrosion methods and by stereoscopic roentgenograms after injection of opaque solutions. These solutions do not usually pass beyond the glomerulus, so they give no information in regard to the postglomerular capillary circulation. The present study is not concerned with this grosser circulation but with the finer distribution of vessels. Recently Lee-Brown,[2,3] by injecting the renal arteries with Berlin blue solution and examining thick sections of the tissue with the binocular microscope, has elucidated many details in this circulation. By this method of using a diffusible pigmented fluid, he traced the path of capillaries which formed a postglomerular anastomosis. Further evidence of such an anastomosis was given by the fact that when the vein of an excised kidney was ligated and Berlin blue injected into one main division of the renal artery, there was a return flow of dye through the other main division of the renal artery. If barium sulphate was injected, no such return flow occurred, because the

particles of barium were caught in the glomerular tuft. This he considers gives further evidence that the anastomosis is a postglomerular one. Microscopically, he locates this anastomosis in the efferent plexus of the cortex and in the medulla, chiefly at the apex of the pyramids. The results of my experiments seem to give further evidence of a postglomerular anastomosis, but one which connects the glomeruli with the extrarenal collateral channels and which permits a circulation to the glomeruli independent of that of the tubules.

The recent experiments of Belt and Joelson [4] in injecting human and dog kidneys deal especially with possible arterial anastomoses after arterial obstruction. They show the constancy of perforating arteries which penetrate through the entire kidney, reach the perirenal tissue and take their course through perirenal fat or form extrarenal anastomoses with branches of the main renal artery or with branches of the renal artery which supply the pelvis and ureter. My experiments would indicate that in the cat after venous occlusion the circulation is effected through such vessels traversing the kidney and penetrating into the perirenal tissue. They would also show that most glomeruli are in free communication with such vessels, since the persistence of glomeruli after venous occlusion indicates that glomeruli commonly have such a close relationship to vessels of this type.

The extrarenal collateral circulation was found by Lindeman [5] and by Barney [6] in experimental hydronephrosis of dogs to consist chiefly of connections with the adrenal, lumbar and spermatic or ovarian vessels. Tuffier and Lejars [7] found in dissecting human cadavers that there were five main groups of anastomosing venous channels, namely, capsular-renal, capsular-mesenteric, capsular-suprarenal, capsular-spermatic and capsular-lumbar. The details of these extrarenal anastomoses are not immediately relevant to my experiments. The injection of the ligated cat kidneys make it evident that some such anastomosis exists, and that the circulation after the obliteration of the renal vein is maintained through already existing channels rather than through newly formed ones. In the present paper I am primarily concerned with the intrarenal circulation which communicates with these extrarenal anastomosing channels, and it has been shown how inadequate this circulation is for the tubules. For the maintenance of the glomeruli, however, it appears to be more than adequate, for when part of the collateral circulation was

cut off by ligating in addition the ovarian or spermatic vessels, the glomerular circulation was still maintained, and the gross and microscopic changes were the same as when the renal vein alone was cut.

How far these results in cats are comparable to the effect of venous occlusion in man is impossible to say, but there is a striking similarity of the gross picture of these experimental kidneys with that of cases of so-called "symmetrical necrosis of the kidney" reported in the literature and associated with pregnancy or occurring in the new-born (Klotz,[8] Bradford and Lawrence,[9] Glynn and Briggs,[10] Torrens,[11] Griffith and Herringham,[12] Jardine and Teacher,[13] Bamforth[14] and others). The gross appearance is complete necrosis of the peripheral portion of the entire cortex with a clearly demarcated hemorrhagic zone in the deeper portion of the cortex. The etiology of the condition is much disputed. Usually it has been attributed to endarteritis, but the evidence is unsatisfactory that this is the causative factor. Torrens attributes the condition to venous thrombosis, a view which Glynn and Briggs challenge, because, they state emphatically, there is no clinical or experimental evidence that occlusion of the renal vein will cause such cortical necrosis. The late microscopic changes cannot be compared because the human lesion is bilateral and rapidly fatal, but Bamforth described in his case degeneration of the tubules while glomeruli appeared normal, and thrombi were seen in many veins while arteries were free.

Summary

1. Ligation and section of the renal vein of one kidney in cats result in congestion and edema followed by a clearly defined zone of necrosis in the periphery of the cortex beneath which there is a zone where tubules are degenerating and glomeruli remain intact.

2. In the later stages, necrosis of tubules is widespread, while many glomeruli persist. The extensive destruction of tubules results in extreme atrophy of the organ which is made up almost wholly of closely approximated glomeruli and capillaries.

3. Injection of the capillary circulation with Berlin blue demonstrates the course of the blood from the main renal artery through afferent glomerular arterioles, then through postglomerular capillaries to the extrarenal anastomosing channels.

4. The persistence of glomeruli with destruction of tubules indicates that there is a certain independence of the glomerular circulation from that of the tubules.

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DESCRIPTION OF PLATES

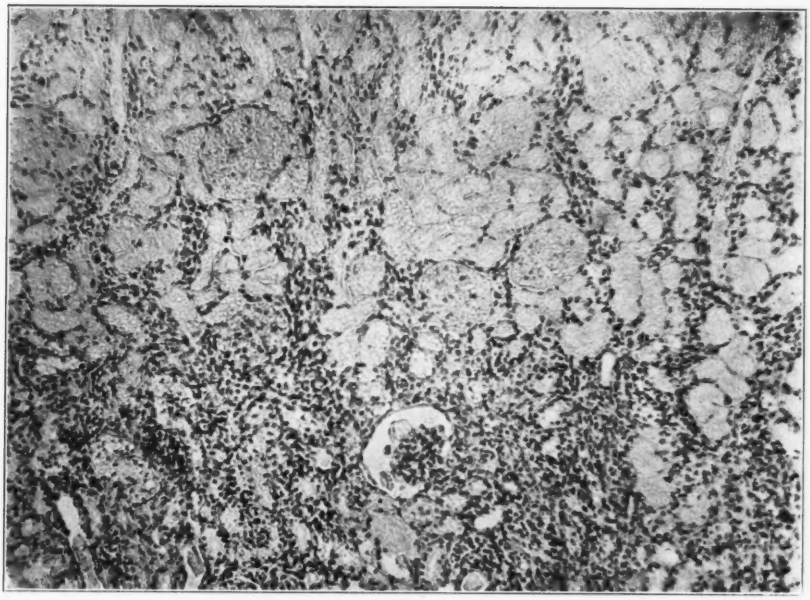
PLATES 16–17

FIG. 1. Kidney of cat, eight days after transecting the renal vein, showing a sharply defined outer zone of complete necrosis, beneath which there is a zone of degenerating tubules. X 100.

FIG. 2. Kidney of cat, five weeks after transecting the renal vein. High power photomicrograph showing that the persisting glomerular tufts are patent to Berlin blue injection fluid. X 500.

FIG. 3. Kidney of cat, two months after transection of the renal vein. Glomeruli have persisted while tubules have atrophied or have completely disappeared. X 250.

FIG. 4. Kidney of dog, three months after transection of the renal vein. The kidney consists entirely of glomeruli and supporting connective tissue, while tubules have completely disappeared. X 50.



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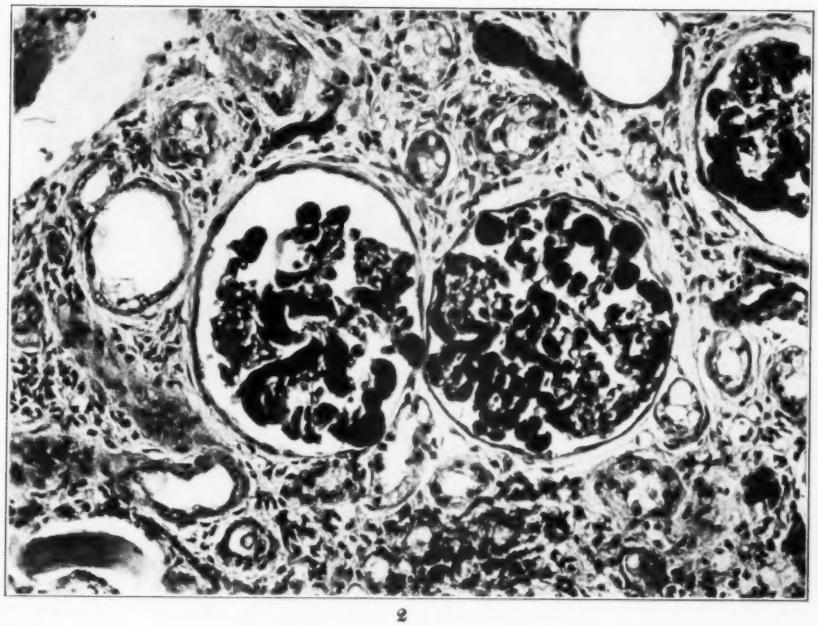

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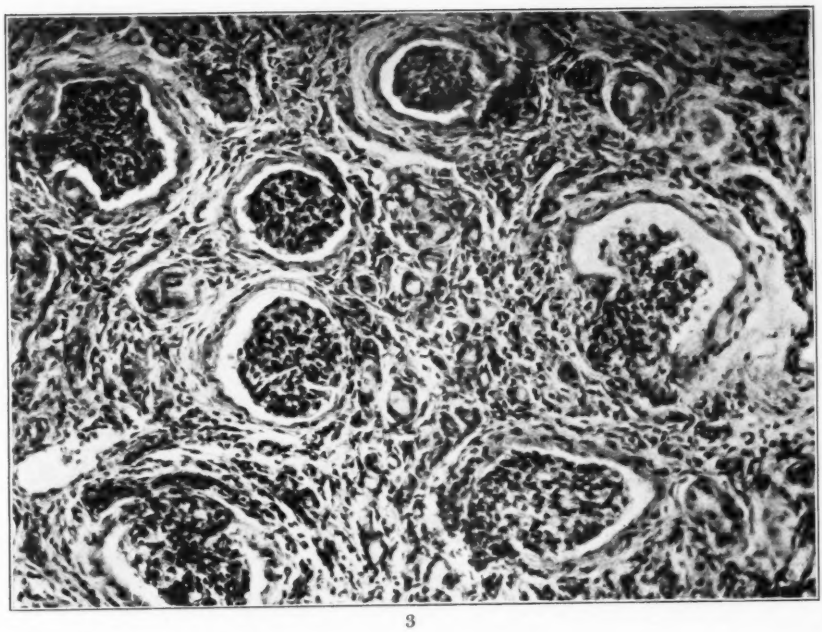

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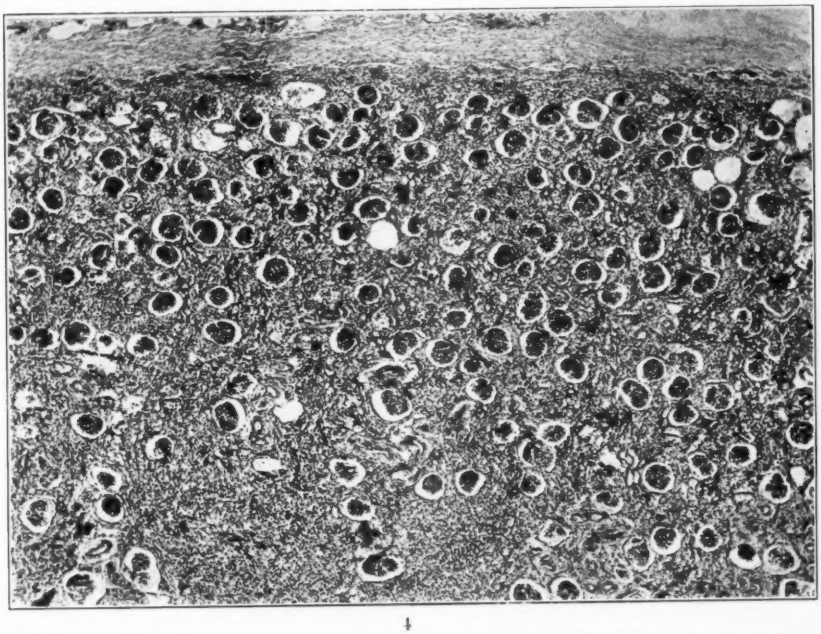

4

Zeckwer The Glomerular Circulation

LIPOID-CONTAINING CELLS IN THE SPLEEN IN DIABETES WITH LIPEMIA *

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In the average spleen removed at necropsy there is little evidence, either gross or microscopic, of lipoid content. However Poscharisky[1] demonstrated fat to be present in spleens removed from cases of many different diseases and to be particularly abundant in those spleens containing amyloid and hyaline masses. This fat is present in small droplets and gives the characteristic staining reactions for neutral fat. He further states that in children the fat is chiefly within the "epithelioid" cells of lymph nodules of the spleen, while in adults it is found in the pulp, trabeculae, capsule and vessel walls.

Kusunoki[2] added further proof of the frequent occurrence of lipoid-containing cells in the spleen, and showed that most of the lipoid was contained in cells of the reticulum. The lipoid gave the following staining reactions: red with Sudan III, black with osmic acid and blue or blue-violet with Nile blue sulfate. Kusunoki also found the number and prominence of the lipoid-containing cells in the spleen to run parallel with the lipoid content of the blood.

Aside from this fairly common finding of lipoid-containing cells in the spleen, there are a few cases recorded in which very marked hyperplasia of these cells occurs, and the lipoid present does not as a rule give the staining reactions usually encountered. These cases are commonly associated with diabetes with lipemia.

The first instance of this striking change in the spleen was mentioned by Coats.[3] The patient suffering from diabetes with lipemia had died in coma. Large lipoid-containing cells made up most of the splenic tissue. Nine other cases have since been reported. The findings in eight of these † are summarized and compared in Table I with those of our three cases.

* Received for publication December 20, 1925.

† One case has been reported by Eppinger, according to Oppenheimer and Fishberg.[4] His publication is not accessible to us.

TABLE I

Comparison of Staining Reactions of Various Fats Found in the Spleen

Case	Type of Lipoid	Sudan III	Scarlet Red	Nile Blue Sulfate	Osmic Acid	Smith-Dietrich	Anisotropic Crystals
I Case No. 1305	1	Red	Red	Blue	Black	+	few
	2	Yellow	Faint	Faint rose	Faint	+	few
II Case No. 1794		Yellow	Faint	Faint rose	Faint brown	+	few
III Case No. 05-172		Orange	Red				
IV Schultze[5]		Faint	Faint	Faint rose	−	+	−
V Lutz[6]	1	Faint		Faint rose	=		
	2	Red		Blue	−		few
	3	Red		Bright rose	Black		
VI Lutz[6]	1	Red		Rose	Black		
	2	Yellow-red		Violet	Black		
VII Marchand[7]		Yellow			Faint		
VIII Williams and Dresbach[8]		+		+			+
IX Smith[9]	1	Red	Red	Blue	Black	+	−
	2	Faint	Faint	Faint rose	Faint	Gray reticulation	−
X Fahr and Stamm[10]		−		−			+
XI Oppenheimer and Fishberg[4]		−				−	

NOTE: In some of the cases there are various types of lipoid present.

Of the three instances of this rare lesion reported in this paper, two were encountered in studying pathologic material placed at our disposal by Dr. Elliott P. Joslin. The third was brought to our at-

tention by Dr. F. B. Mallory. This last case has not been studied as carefully as we should wish since the necropsy was performed twenty years ago and the material other than the slides has been lost. However, we are particularly fortunate in that our first two cases were under careful observation for five and eight years respectively.

Case Histories

Case I. No. 1305. White male. Onset of diabetes at 10 years and 6 months of age. Maximum body weight (clothed) 75 lbs. (12 per cent above normal). The illness lasted five and one-half years. The last few weeks he received small amounts of insulin but died in coma.

Table II

Blood Changes in Case I, No. 1305

Date	Blood Sugar gm. per 100 cc.	Blood Fat * per cent	
May 22, 1917	0.09	..	
Nov. 17, 1919	0.21	..	
Dec. 1, 1919	..	0.51	
June 3, 1921	0.25	1.00	
Jan. 3, 1922	0.24	1.68	
Feb. 13, 1922	0.22	1.72	
Aug. 9, 1922	0.20	0.92	Given small amounts of insulin
Aug. 28, 1922	0.21	1.61	
Sept. 3, 1922	..	1.54	
Oct. 20, 1922	..	2.56	During coma
		2.30	
		1.80	

* Analyses by Bloor's [11] total fat method. (Normal 0.75 per cent).

In Table II are given a few selected blood sugar and blood fat analyses which serve to show the general character of the blood changes.

Necropsy Report: Age 16 years. Necropsy one hour postmortem. Limited to upper abdominal incision. The body is that of a fairly well developed and nourished white boy. No edema. No jaundice.

Peritoneal Cavity: Surfaces smooth and shining. No free fluid.

Pleural Cavities: Negative.

Pericardial Cavity: Negative.

Heart: Normal size. No gross evidence of fibrosis. Valves negative. The coronary arteries are negative.

Lungs: Soft, crepitant except at bases, which are somewhat soggy. On section both bases are moist and reddish, with fluid in bronchi.

Spleen: Not remarkable.

Pancreas: Small.

Gastro-Intestinal Tract: Negative.

Liver: Not remarkable. The bile ducts are patent. The gall bladder is negative.

Kidneys: Negative.

Aorta: Scattered atheromatous plaques.

Anatomic Diagnoses: Hypostatic congestion of lungs, early arteriosclerosis.

Microscopic Findings.

Heart: Moderate amount of subepicardial fat. There is a slight increase of fibrous tissue.

Lung: Congested. A few polymorphonuclear leucocytes and red blood cells, together with endothelial leucocytes containing pigment, in alveoli. A few polymorphonuclears and lymphocytes in submucosa of bronchi. Moderate amount of carbon pigment beneath pleurae and about larger vessels and bronchi.

Spleen: Large vacuolated cells resembling endothelial leucocytes are present in the lymph nodules and in the splenic pulp. These cells are most prominent in the outer portions of the lymph nodules (Fig. 1). Some lymph nodules have reddish hyaline material in their centers, and a few contain scattered phagocytic endothelial leucocytes. Moderate degree of hyaline thickening of walls of arterioles.

With fat stains on formalin-fixed tissue preserved for three years, there appear to be two types of fat present (Table I, Case I). The first type, occurring in the larger globules found among the cells in the outer part of the lymph nodules as a rule, stains deep red with Sudan III and scarlet red, blue with Nile blue sulfate and brownish black with osmic acid. The second type occurs in smaller droplets in endothelial leucocytes both in the splenic pulp and to a less extent in the lymph nodules, as well as in the hyaline substance noted above and occasionally free in the neighborhood of small blood vessels. These droplets stain yellow-red with Sudan III, a faint red with scarlet red, very faint rose with Nile blue sulfate and pale gray with osmic acid. Both types of fat stain with the Smith-Dietrich method. With the polariscope when the slide is warmed, a few

anisotropic crystals are detected, which disappear with further heating but reappear on cooling. These findings are summarized in Table I (Case I).

Pancreas: Congested. Slight increase in connective tissue of islands. Moderate increase in interlobular connective tissue.

Liver: Fine vacuoles suggesting fat occur in the endothelium of the sinusoids. These stain red with Sudan III and scarlet red and brownish black with osmic acid. The liver cells are vacuolated especially about the central veins, and give similar staining reactions.

Kidneys: Not remarkable.

CASE II. No. 1794. White female. Onset of diabetes at 25 years of age. Body weight at onset 130 lbs. Duration of disease eight years. On June 6, 1920, blood sugar was 0.25 gm. per 100 cc. and blood fat 1.10 per cent. On May 21, 1921, blood sugar was 0.20 gm. per 100 cc. and blood fat 0.80 per cent. In June, 1922, she weighed when clothed 71 lbs. Under insulin treatment she gained weight and strength and in June, 1925, shortly before her death weighed 135 lbs. After breaking diet, she became suddenly ill with severe pain in the left chest and arm. She had five Stokes-Adams seizures, and died after a few hours from cardiac failure.

Necropsy Report: Age 33 years. Weight 130 lbs. Necropsy one hour postmortem. The body is that of a well developed and nourished white female. No edema. No jaundice. Abdominal fat layer one and one-fourth inches thick.

Peritoneal Cavity: Surfaces smooth and shining. No free fluid.

Pleural Cavities: Negative.

Pericardial Cavity: Negative.

Heart: Weight 315 gm. Epicardium negative. Considerable subepicardial fat. Apex of left ventricle yellowish gray, opaque and separated from normal myocardium by thin red zone. On section, lower two-thirds of interventricular septum is similar in appearance. A large thrombus is adherent to the endocardium. This entire region stains intensely red with scarlet red. In the endocardium just below the aortic ring are yellowish raised plaques which also surround the coronary orifices. The right coronary artery shows numerous atheromata; both branches of the left are tortuous and sclerosed. The first portion of the left barely admits a horsehair.

Spleen: Somewhat grayish. Only a small amount of pulp can be scraped away.

Pancreas: Weight 60 gm. Negative in gross.

Liver: Weight 1835 gm. Yellowish red. Bile ducts are patent. Gall bladder is distended with bile.

Kidneys: Weight 350 gm. Negative in gross.

Adrenals: Negative.

Bladder: Negative.

Genitalia: Negative.

Aorta: Numerous atheromatous plaques.

Anatomic Diagnoses: Infarct of heart; coronary sclerosis; and arteriosclerosis.

Microscopic Findings.

Heart: Moderate ingrowth of fat. Numerous hemorrhages into myocardium with polymorphonuclear leucocytes between the fibers. In several foci, muscle fibers have lost their striations and normal staining reaction, and have granular or hydropic cytoplasm. Numerous vessels are plugged with fibrin the meshes of which contain red blood cells and polymorphonuclear leucocytes. In one place there is marked fibrous thickening of the endocardium with hemorrhages beneath. Closely attached to the endocardium are masses of fibrin with red blood cells and polymorphonuclear leucocytes enmeshed. The coronary arteries show marked thickening of the intima due to collections of vacuolated and pigmented endothelial leucocytes. When stained for fat, the material in these cells is shown to be similar to that in the spleen.

Lungs: Edema. Carbon pigment is present beneath pleura and about larger vessels.

Spleen: Congested. Numerous plasma cells occur throughout. A large number of endothelial leucocytes filled with fat droplets are distributed throughout the pulp and are particularly abundant in the germinal centers (Fig. 2). Some of the cells contain numerous droplets (Fig. 3), while others are greatly distended by one large droplet (Fig. 4). The large ones are apparently due to the coalescence of droplets originally contained in endothelial leucocytes but set free by degeneration of the cells. The germinal centers are few and most of them contain masses of hyaline material. One contains a network of fibrin.

The results obtained with fat stains (on tissue fixed in formalin for five months) are summarized in Table I (Case II). In this spleen only one type of fat appears to be present. It stains yellow with Sudan III, faint yellow-red with scarlet red, very pale rose with Nile blue sulfate and very pale brown with osmic acid. Osmic acid appears to stain only the periphery of the globules, but they stain well with the Smith-Dietrich method. The polariscope reveals a few anisotropic crystals when the section is warmed, which disappear on further heating and reappear on cooling.

Pancreas: There is slight increase in the interlobular connective tissue. The islands appear to be much fewer than normal. Islands can be found showing varying degrees of sclerosis, from those apparently normal to practically complete disappearance of island cells.

Liver: The periportal areas contain numerous polymorphonuclear leucocytes, lymphocytes and plasma cells. Rarely a few of the liver cells at the periphery of the lobules are necrotic and surrounded by endothelial and polymorphonuclear leucocytes. Bile stasis is apparent in some portions. Endothelial leucocytes in the sinusoids are phagocytizing pigment, and some contain fine droplets. When stained for fat, these droplets are yellow with Sudan III and pale violet with Nile blue sulfate.

Kidneys: The glomeruli are markedly congested. The tubules are edematous. In Henle's loops the epithelial cells have lost all substance except for the nucleus and are greatly distended.

Aorta: There is very marked fibrous thickening of the intima in which are many endothelial leucocytes containing fat droplets. A few lymphocytes are also present. The vessels of the media are surrounded by an increased amount of fibrous tissue, with moderate infiltration by endothelial leucocytes. The droplets in the endothelial leucocytes when tested for fat are stained yellow with Sudan III and blue-black by the Smith-Dietrich method. With polarized light the droplets are found to be anisotropic.

CASE III. No. 05–172. White male, 21 years of age. He was admitted to the hospital in deep coma and with a marked odor of acetone on the breath. No history could be obtained and death occurred in thirty hours. The urine showed a large amount of sugar and of diacetic acid, as well as a slight trace of albumen.

Necropsy Report: Age 21 years. Examination twenty-nine hours postmortem. The body is that of a well developed but poorly nourished white male. The significant findings in the necropsy may be summarized as follows.

Heart: The heart's blood on standing becomes covered with a creamy, bluish white scum. When the blood was placed in a cylinder this layer occupied the upper three-fifths of the column. The myocardium shows small scattered foci which are slightly darker than the surrounding tissue, translucent and slightly depressed.

Spleen: Weight 320 gm. On section it is dark red, firm, with markings distinct. A very small amount of pulp comes away on scraping.

Pancreas: Weight 45 gm. Negative in gross.

Liver: Weight 2045 gm. The capsule is smooth. The tissue is brownish red. The markings are indistinct. Occasionally foci throughout the liver, each involving several lobules, are paler and more yellowish.

Kidneys: Weight 360 gm. The capsule is stripped readily, leaving a smooth surface. On section the kidneys are reddish gray, congested and the markings are distinct.

Aorta: There are a number of opaque yellow raised plaques in the intima of the ascending portion of the arch. These are fewer in the remainder of the aorta, and they tend to cluster about the mouths of the intercostal arteries.

Microscopic Findings.

Heart: Sections of formalin-fixed material stained with scarlet red show fat droplets in the muscle fibers. The vessels contain much finely divided fat.

Spleen: There is moderate congestion. The meshes of the reticulum are filled with large foamy cells. When sections of formalin-fixed material are stained with scarlet red, these cells contain orange masses. The vessels contain much finely divided fat. In some of the nodules, the endothelial cells show evidence of proliferation. There is an increased number of eosinophiles.

Pancreas: Sections of formalin-fixed tissue stained with scarlet red show a considerable amount of finely divided fat in the epithelium of the acini and of the islands. The larger vessels show much finely divided fat.

Liver: There is considerable congestion. The nuclei are vacuolated, giving the typical appearance of glycogenic infiltration. The endothelial cells lining the sinusoids are somewhat enlarged and finely vacuolated. Sections stained with scarlet red show fine fat droplets evenly distributed throughout the liver cells and the endothelial cells.

Kidneys: The epithelium of the tubules is markedly vacuolated. In sections of formalin-fixed tissue stained with scarlet red, these vacuoles are seen to be filled with fat except in the region of Henle's loops. The character of the vacuolization here suggests glycogenic infiltration. There is also finely divided fat in the glomeruli and in the blood vessels.

Adrenals: The formalin-fixed sections stained with scarlet red show a marked deposit of fat toward the periphery.

Lymph Node: The sinuses are dilated and filled with vacuolated endothelial cells, some of which are in mitosis. In some of the lymph nodules there is an increased number of endothelial cells at the center. The endothelial cells contain finely divided fat.

Discussion

Although the significance of large numbers of lipoid-holding cells in the spleen is not clear, the rarity of the condition makes it seem worth while to record these three cases. So far as can be judged from the staining reactions, the lipoid is present in various forms and sometimes even varies in the same case, though cholesterol esters or related substances predominate. Thus in Case I, the larger droplets are presumably soaps or fatty acids among which, according to the reaction with osmic acid, are unsaturated fatty acids. The smaller droplets contain cholesterol esters.

In our first two cases, the long duration of the diabetes apparently has some relation to the condition. Two other cases of diabetes, about the same age as Case I, also died in coma. Their disease was of comparatively short duration, and the lipoid-containing cells were not found in the spleen, nor was there any evidence of lipemia, though complete necropsies were done. In Case II, the blood fat was abnormally high five years before death. The gain in weight from 71 to 135 pounds under insulin treatment and the amount of body fat found at necropsy suggest that more frequent analyses of

the blood would have revealed a more marked lipemia. In Case III, no history could be obtained, but the patient died in coma, with large amounts of sugar in the urine, and a striking lipemia was found at necropsy.

The spleen is not the only organ involved in this condition, though there the changes are more striking than elsewhere as a rule. Thus in Case I, lipoid is present in the spleen, the endothelial cells of the liver sinusoids and the intima of the aorta. In Case II, lipoid-containing endothelial cells occur in the spleen, liver, coronary arteries and aorta. The lymph nodes and bone marrow were not examined in either case. The fat is widely distributed in Case III, being present in the heart muscle, the parenchymal cells of the pancreas and liver and the renal epithelium, while the entire reticulo-endothelial system of the spleen, lymph nodes and liver contains lipoid material.

Oppenheimer and Fishberg [4] have stressed this involvement of the reticulo-endothelial system in lipemia and regard this group of cells as very important in relation to lipoid metabolism. Very similar changes to those in the human cases were produced in rabbits by Anitschkow [13] through feeding cholesterol dissolved in sunflower oil.

One of the puzzling features of this condition is the variation of the chemical constitution of the lipoid taken up by the cells of the reticulo-endothelial system. Of course the staining reactions are not entirely reliable criteria, but in several of the cases of this condition there is a marked difference in the reactions of the lipoid material in the circulating blood and that present in the reticulo-endothelial system. The selective absorption of cholesterol compounds and phosphatides seems not impossible.

Hyperplasia of lipoid-containing cells in the spleen has been observed in a few instances not associated with diabetes and lipemia. We encountered this condition in a negro 35 years old who died of an acute exacerbation of subacute pancreatitis. Smith [9] reported four instances of lipoid cell hyperplasia in infants and Siegmund [14] has reported one.

In the cases of diabetes with lipemia considered in this paper, there is another element of interest. Vascular lesions are very common. Whether these are merely xanthomas occurring in the intima of the vessels instead of the skin or definite atheromatous plaques is

not easily decided. The case reported by Oppenheimer and Fishberg [4] was a girl of 6 years, in whom yellowish patches occurred in the intima of the aorta and in the endocardium. Both patients reported by Lutz,[6] aged 53 and 36 years respectively, showed atheromatous patches in the aorta. The aorta of Smith's [9] case, a man of 22 years, contained atheromata. In the other reported instances the condition of the vessels was not noted. Atheromata were found in the aorta of our Case I, aged 16 years. Our Case II died of a cardiac infarct resulting from coronary sclerosis at the age of 33. The aorta also was markedly sclerosed. The lipoid present in the endothelial cells in the vascular lesions gave the same staining reactions as that in the cells in the spleen. Case III, 21 years of age, showed numerous yellowish, raised patches in the intima of the aorta.

Here then are five persons from 6 to 33 years of age, with lipoids in the blood that are taken up extensively by the cells of the reticulo-endothelial system, and all five show vascular changes.

There may well be a real danger in permitting patients to live on a diet of high caloric value with the aid of large amounts of insulin. The considerable intake of food above energy requirements, as shown by marked and often rapid gain in body weight, must result in large increase in the amount of lipoid in the circulation. While it is not proved that lipemia is harmful, these few cases suggest that there may be some relationship between it and arterial disease. In a condition such as diabetes, which seems to predispose toward arteriosclerosis, any factor which might tend to bring about vascular change should be avoided.

Summary and Conclusions

1. Three cases of diabetes mellitus with large lipoid-containing cells occurring in the spleen are reported and compared with eight others collected from the literature. Lipemia is present in all the cases except that of Fahr and Stamm.[10]

2. The lipoid material in the cells, so far as can be judged from staining reactions, consists of cholesterol esters or related substances, or phosphatides. Fatty acids or soaps may also be present.

3. The reticulo-endothelial system is probably involved in lipoid metabolism, and may perhaps selectively absorb cholesterol compounds or phosphatides.

4. Arteriosclerosis appears to be associated with this condition. Lipemia, produced by a high caloric diet, by a poorly balanced diet or by the general cachectic state of the tissues in severe diabetes, may predispose to atheromatous degeneration in the arteries.

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DESCRIPTION OF PLATES

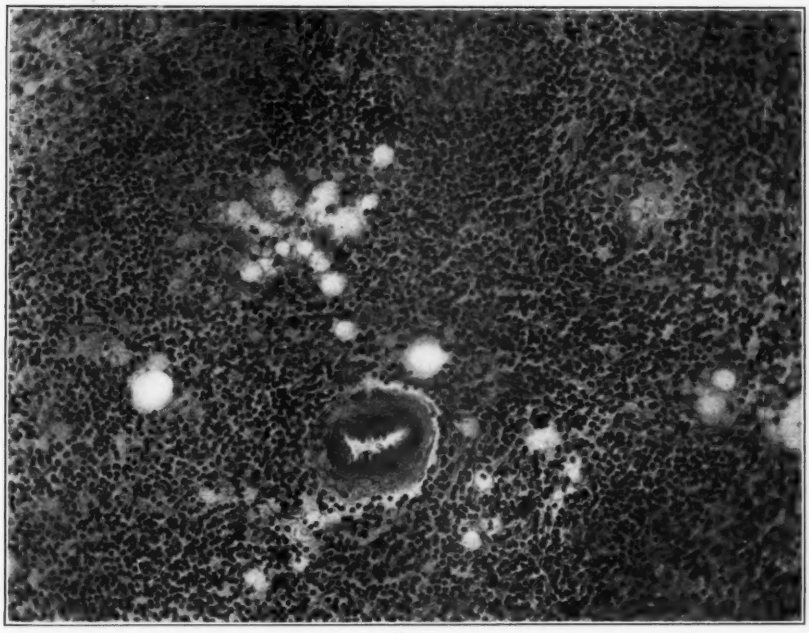
PLATES 18–19

FIG. 1. Case I. Showing distribution of lipoid-containing cells in the spleen, and also hyaline material in the lymph nodules. X 100.

FIG. 2. Case II. Showing distribution of lipoid-containing cells in the spleen. X 100.

FIG. 3. Case II. Lipoid-containing cells in the spleen. X 500.

FIG. 4. Case II. Lipoid-containing cells and a large fat droplet formed by coalescence of smaller ones. X 500.



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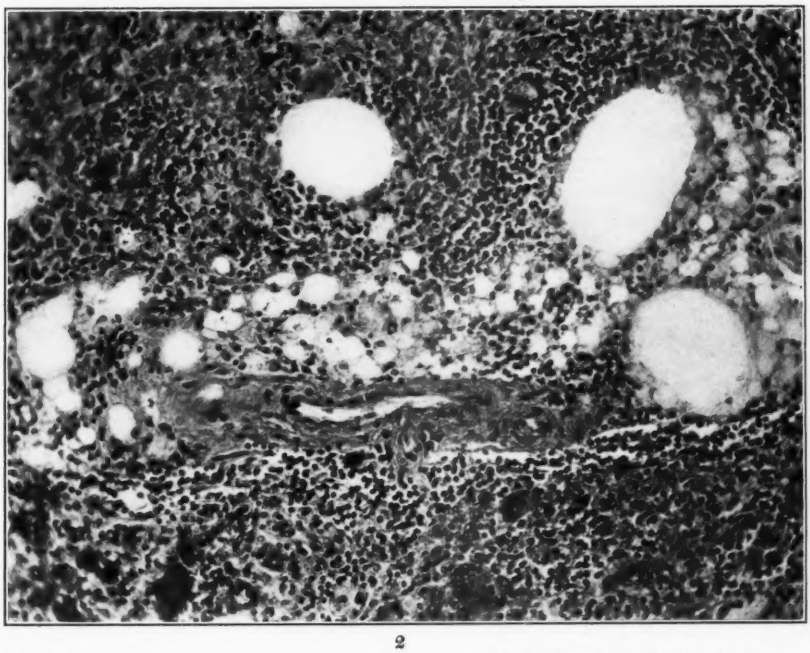
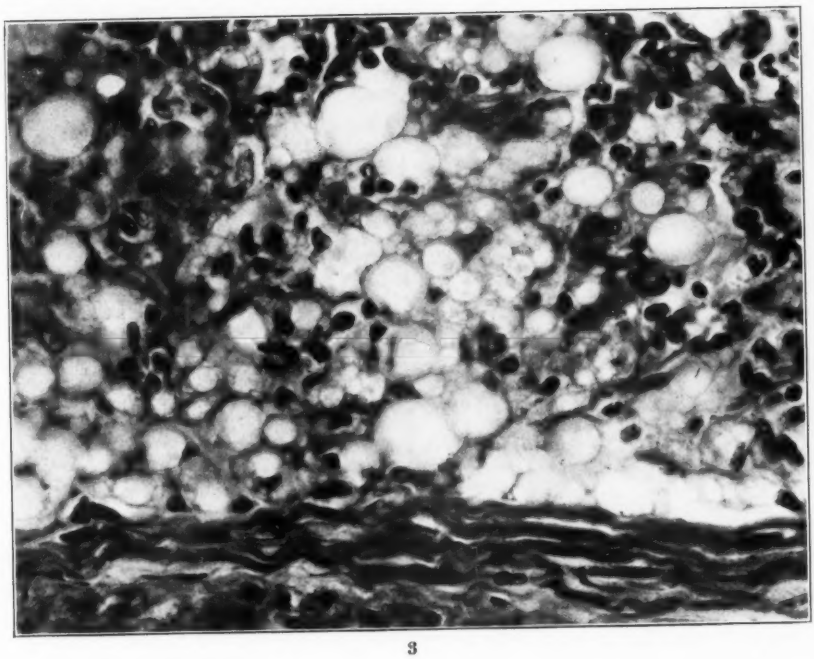


2

Warren and Root Lipoid-Containing Cells in the Spleen



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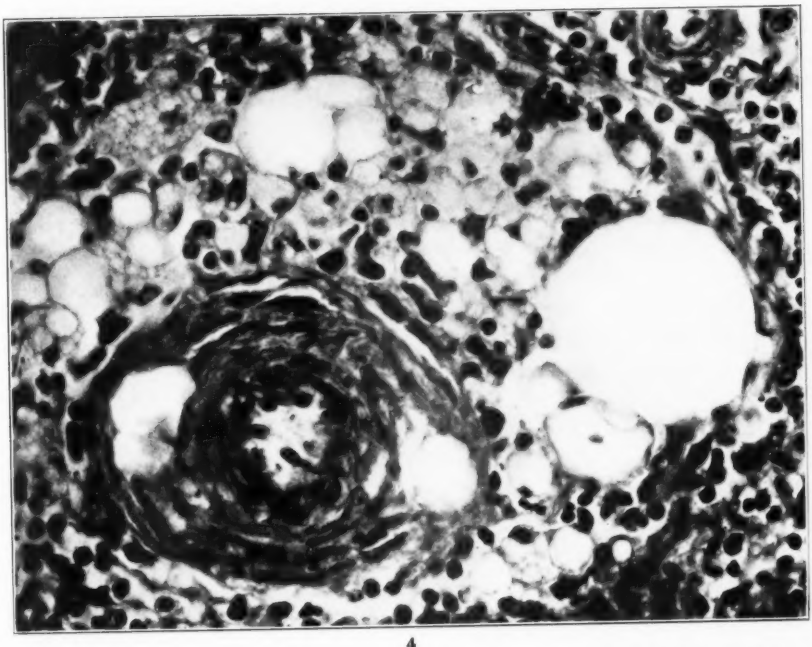

4

Warren and Root

Lipoid-Containing Cells in the Spleen

PRIMARY GELATINOUS CYLINDRICAL CELL CARCINOMA OF THE LUNG *

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Primary papillary cystadenoma of the lung is a rare tumor. Helly [1] and Löhlein [2] described such growths. Nicholson [3] found tumors of the lung of similar structure to be metastases from an apparently benign ovarian tumor. Cylindrical cell carcinoma of the lung with papillary and cystic formations has been described by several authors (Ribbert,[4] Briese,[5] Kretschmar[6] and Gordon[7]).

Helly [1] reported a distended emphysematous lung, studded with nodules of various sizes, containing granular stringy coagulated masses. The normal alveolar epithelium was replaced in the nodular areas with high columnar epithelial cells many of which were goblet cells. Adjacent alveoli were sometimes partially replaced with this type of epithelium. There was always a sharp demarcation between the normal alveolar epithelium and the high columnar cells of the tumor. The alveolar septa were not destroyed and the bronchi, bronchioles and blood and lymph vessels were not invaded. A clear line of demarcation between the tumor nodules and the surrounding tissue was not found. Helly considered this tumor to be a benign cystadenoma of the lung, starting from the transitional epithelium between the bronchioles and the alveoli. Löhlein [2] described a tumor in the right lower lobe of the lung, the size of a fist and gelatinous in consistence. Bronchi, bronchioles and lymph nodes were normal. The edge of the tumor mass was composed of cylindrical mucus-secreting epithelium which replaced the normal lining of the alveoli. In the center, the alveolar septa had been replaced by large cysts filled with tenacious mucous material. Pseudopapillary formations were observed. The transition between the neoplastic alveolar epithelium and the normal lining of the bronchi was not always distinct. Löhlein considered this to be a benign cystic tumor starting from alveolar epithelium. Sternberg men-

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tioned in the discussion of this case a tumor of similar structure with brain metastases.

Nicholson [3] described tumors of the lung of similar structure, but he showed these to be metastases from a pseudomucinous cystadenoma of the ovary. Pick,[3] in a critical discussion of Nicholson's paper, Moise,[8] Adler [9] and others have discussed in detail the various interpretations that can be placed upon the origin of the primary tumors of the lung.

Report of Tumor

Some lung tissue was received from the surgical clinic (Auguste-Viktoria Krankenhaus, Berlin-Schöneberg) which seemed to show changes similar to those described by Helly, Löhlein and Nicholson. It was impossible to determine whether this tumor was primary or secondary from such a specimen. The patient died several months later and at necropsy it was found to be a primary lung tumor.

The surgical specimen was a piece of tissue the size of a hen's egg, rather dense with fibrous tissue and an adherent, grayish white, gelatinous substance. Cystic areas filled with opaque mucous material were scattered throughout the denser tissue.

Microscopically, this specimen is composed of fibrous tissue, fat and striated muscle. Elastic connective tissue stain brings out the elastic membrane of the pleura, which is split into layers by fibroblasts. Cystic areas visible in this connective tissue layer are lined with high columnar or goblet cell epithelium and contain material giving the reaction for mucin. True papillary formations can be observed. Delicate incomplete connective tissue septa between cysts show evidence of a confluence of several cysts. The loose connective tissue around some of these cysts is infiltrated with tumor cells and contains coagulated gelatinous extracellular substance (Fig. 1).

Adherent to the inner side of this fibrous layer is tissue which resembles, with low magnification, an emphysematous lung. Thickened connective tissue septa with perivascular round cell infiltration separate the lobules. The delicate alveolar septa are intact and the elastic tissue framework is shown by special staining to be normal in distribution. The distended alveoli are lined with regular, high cylindrical epithelium; goblet cells are present in places. In some few areas the normal cuboidal cells remain intact. The lining is

always in a single layer; transitional forms between the high columnar tumor cells and low cuboidal alveolar epithelium are not observed. Papillary growths into the distended alveoli or cysts are frequently seen. These alveoli are distended with a mucous mass which is a secretion of these lining tumor cells. Desquamated tumor cells are found in the lumen of the cysts, some with two or even more nuclei.

The ciliated cylindrical epithelium of the bronchi and terminal bronchioles is well preserved and not displaced by tumor cells. There is always a sharp line of demarcation between the deeper staining, ciliated epithelium of the bronchioles and the lighter staining, high cylindrical tumor cells of the alveoli (Figs. 2 and 3).

Résumé of Necropsy Findings

Mediastinum: Massed and adherent lymph glands on left side from hilum to level of first rib. These are hard and grayish white and on section show many cysts filled with tenacious, whitish mucus.

Right lung: Negative.

Left lung: Pleura firmly adherent. Lung solid and firm in consistence. No normal lung tissue found upon section. The lung is filled with grayish white, confluent nodules. Tissue between these nodules has a glass-like translucency and is composed of a delicate network with mucous cysts the size of a pin-head and larger. A tenacious mucus exudes from the whole cut surface. The bronchi are uniform in thickness and normal in appearance. The hilum glands are of the same consistence as the lymph glands of the neck.

Abdominal organs: Negative. The ovaries are entirely normal.

Microscopic Examination

The histologic findings are the same as those mentioned in the surgical specimen. The walls of some of the larger bronchi examined show an invasion by the tumor cells with complete destruction of the mucosa. The peribronchial lymph spaces and mucous glands are invaded and destroyed by the neoplastic growth. The hilum glands are completely replaced by tumor cells which form cysts and small and large groups of cells surrounded by mucous material. We consider this tumor to be a primary gelatinous cylindrical cell carcinoma of the lung.

We found primary carcinoma of the lung or the bronchi to have occurred in 33 of about 5000 necropsies between 1911 and 1921, or in 0.67 per cent. Older statistics mention a much lower percentage: Kaufmann [10] states 0.2 per cent in his textbook; Ewing,[11] 0.1 per cent; Briese,[5] 0.46 per cent; Barron,[12] 0.29 per cent during 1899 to 1921, but only for the years 1919 to 1921, 0.9 per cent. Recent publications of European investigators show an increase in the frequency of the lung carcinomas in the last years, similar to that observed by Barron for this country. Berblinger [16] noted a frequency of lung carcinomas in 6.6 per cent of all observed carcinomas in 1920, but of 11.11 per cent in 1923; Seyfarth [18] reported 6.23 per cent in 1920 and 15.5 per cent for the first half year of 1924; Lubarsch stated 5.4 per cent in 1920–21; Stähelin [17] found 2.19 per cent in 1900, against 4.9 per cent in 1924; and Kikuth [19] mentioned 1.7 per cent in 1897, but 9.4 per cent in 1923, adding that he could not state an increase of the absolute frequency of the carcinomas during this period in his material.

The indisputable increase of the lung carcinomas during the last twenty years, especially the last five, is the source of a lively discussion in the recent literature about its suspected causation. We may classify the different theories about the subject according to the type of the suspected causative factors and place them in three groups.

The first group may comprise the bacteriotoxic causes. A well known source of bronchial carcinoma is the metaplastic processes in the lining of old tuberculous cavities and syphilitic scars. The frequent occurrence of similar metaplasias in the bronchial mucosa, described by Askanazy [18] as precancerous lesions, after an influenza affection led several investigators (Moise,[8] Berblinger,[16] Läschke [20]) to suspect a correlation between the recent influenza epidemic and the marked increase of the lung carcinomas in the last years. But their conclusions show many weak points and are in the final analysis not very convincing. First, we miss a similar sudden increase of the lung carcinomas after the influenza epidemic in the years 1890 to 1894; second, the increase started in a minor degree already before 1920 as shown in the statistics of Stähelin,[21, 25] and Hampeln [14] comparing the data noted for the years before and after 1900 (0.1 to 0.2 per cent against 0.4 to 0.6 per cent); third, even the authors in favor of this theory could not prove the occurrence of a

previous "influenza," a diagnosis which was quite misused at that time, in the majority of their cases (Läschke [20] in less than 50 per cent). Other investigators of the same question (Gottstein, Stähelin, Kikuth, Hampeln) were unable to demonstrate this connection in a noteworthy percentage; fourth, the contrast in the male and female rates (Seyfarth 5.3: 1; Berblinger 4.25: 1 (for 1920 to 1924 only 2.7: 1); Kikuth 1.8: 1) is an important objection against this theory (Mathias [23]).

The causes classified in the second group are those of physical means. A trauma of the chest is too rare a cause of a lung carcinoma to have any relation to the increasing frequency of these tumors. Also the suspicion mentioned by Kikuth that the increasing use of X-rays for the treatment and diagnosis of thorax diseases might have some connection with the increase of the lung carcinomas seems to be unjustified because the frequency of the lung carcinomas does not correspond to the increase in the use of X-rays, and even Kikuth could not establish this connection in his material. Furthermore we may eliminate the inhalation of cigarette smoke as a causative factor for this increase, as stated by Fahr [23] who based his theory on the increased habit of cigarette smoking and the predominance of the affection in the male sex. Cigarette smoke may have only a contributory influence if any at all. A theory of more importance is supported by Hampeln.[14] He believes that a certain relation exists between the increased production of smoke and dust in the big cities which are substances causing by continuous inhalation a chronic irritation of the bronchial and lung epithelium, and the increasing frequency of the lung carcinomas. This theory, like that concerning the influenza epidemic, seems to be quite acceptable at first view, but the investigations of other authors (Dynkin,[21] Sachs,[25] Berblinger [16]) failed to substantiate this presumption. They were unable to state a higher frequency of carcinoma in pneumonoconiotic lungs than in other lungs. Schmorl [22] denies also a causative connection between the well known lung carcinoma of the Schneeberg miners with a primary pneumonoconiosis. He is rather convinced that these tumors are caused by the chemical effect of inhaled gaseous substances and suspects that arsenic compounds have to be made responsible for the frequent development of carcinomas in the lungs of these miners.

This explanation brings us to the last and third group of causa-

tive factors, those of chemical nature. We can eliminate from the list of the possible or suspected chemical factors which may be responsible for the increasing frequency, all those which have no generalized distribution and to which we are exposed to a certain degree for a period longer than the last twenty years. This consideration excludes the theory of Kraus [23] proposing a relation of the gas poisonings during the war with the lung carcinomas. By far the majority of the cases were never exposed to this injury. Furthermore, we can neglect the inhalation of acids and alkalis as unimportant in this question. A theory which fulfills the above mentioned conditions was expressed by Stähelin.[17] According to him the main factor responsible for the increase of the lung carcinomas in the last fifteen to twenty years is not the inhalation of smoke dust but the inhalation of dust containing chemical substances which possess a specific carcinoma-producing quality. He believes that the small tar and oil particles in the dust of tarred or oiled roads and the oxidation products of gasoline and benzol, daily inhaled in large amounts, are the causative factors for the increase.

His theory is based on the following viewpoints: (1) the tarring of the roads and the more general use of gasoline for automobiles date back about twenty years and have increased rapidly since that time; (2) tar products are recognized as carcinoma-producing substances in case of chronic application; and (3) carcinoma of the lung in rabbits and guinea-pigs was produced successfully by injection of tar products in the trachea (Kimura [24]). This theory is based on several very important facts and is worth examining on a broad experimental and statistical basis. Its practical importance is obvious in a country which has the most tarred roads and automobiles in the world.

Histologically there are three origins of the primary carcinomas of the lung: the epithelium of the bronchi, of the peribronchial mucous glands and the respiratory epithelium of the alveoli. Very briefly the characteristic features of these three types of carcinoma are as follows: carcinoma arising from the epithelium of the bronchi usually starts in the large bronchi, seldom in the bronchioles; it invades the lung along these bronchi from above downwards; and the cells are either cylindrical or squamous in outline. The former may form glandular cysts and at times mucus may be found.

The squamous cells are metaplastic bronchial epithelium. Car-

cinoma arising from the peribronchial glands invades the submucosa of the bronchi and replaces the bronchial mucosa extending down into the alveoli and adjacent structures; the lymph spaces and adjacent interlobular septa are invaded, with destruction of the lung architecture; and histologically the cells may resemble very closely those described in our case, but there is always a loss of the adenomatous structure in many places with solid and scirrhus carcinomatous changes possessing marked infiltrative power. Carcinoma arising from the pulmonary epithelium resembles grossly pneumonic infiltration. Histologically the cells are flat, cuboidal or cylindrical; the alveoli are lined or filled with tumor cells with sometimes a tendency to papillary formations; goblet cells with mucous secretion are characteristic for the cylindrical cell type; bronchial and bronchiolar epithelium does not show neoplastic changes; and the septa between the alveoli are not invaded by the tumor cells.

The carcinoma described in this report belongs to the primary pulmonary epithelial variety. The alveoli are well preserved and lined with tumor cells. The epithelium of the air passages is normal and does not participate in the carcinomatous growth. All adenomatous growths are lined by the same type of cells. There is no invasion of the alveolar septa.

Ribbert,[4] Briese,[5] Kretschmar [6] and Gordon [7] have each described a tumor similar to the one we are reporting here and have interpreted it as a primary carcinoma of the lung starting from the alveolar epithelium.

The question of single or multiple origin of such a tumor has been discussed by these authors. They concluded that there is either a diffuse genesis (Kretschmar, Gordon) or a multiple origin with the neoplastic changes occurring independently in many different foci (Briese, Ribbert). The multiple confluent nodules in the left lung of our case, without evidence of a primary focus, has lead us to the conclusion of the multiple origin of this tumor.

The mucus could probably serve as a medium of transporting carcinomatous cells from one alveolus to another within a limited area. The cells of typical adenocarcinomatous areas in the bronchial mucous membrane could be transported through the same medium and these areas would serve as foci for spreading to the alveoli of a wider area. We have mentioned that there was no invasion of the lymph spaces between the alveoli, and consequently

we think this was hardly an avenue for metastasis. The large lymph vessels along the bronchi and in the pleura showed adenocarcinomatous cysts. We could find no evidence of metastasis by the blood stream.

The tumors described by Helly and Löhlein were interpreted by them as benign adenomas based upon their non-metastasizing and non-destructive features. This can hardly be considered as conclusive evidence in view of the histologic nature of the neoplasms. It has been shown that the primary cylindrical cell carcinoma starting from the alveolar epithelium as described by Ribbert, Kretschmar, Briese, Gordon and by us does not destroy the alveolar structure of the lung and does not metastasize in all cases (Ribbert, Kretschmar, Gordon). Also we think that Pick's interpretation of Helly's tumor as a metastatic growth, because of its distribution throughout the lung in nodular formation, is hardly correct. We, as well as Ribbert and Briese, have observed the genesis of such adenocarcinoma from many different centers in the lung. Moreover, there was no clear line of demarcation between tumor tissue and lung tissue in these cases. Therefore, we conclude that these tumors are not benign adenomas but primary cylindrical cell carcinomas which started from the alveolar epithelium.

Finally, a short note may be included about the clinical difficulty in diagnosis between carcinoma and tuberculosis of the lung. It is known that sometimes a primary carcinoma of the lung starts in an old tuberculous cavity and that the physical and X-ray findings may be very similar in these diseases. But there is another source of wrong diagnosis. The finding of acid-fast bacilli in the sputum is usually considered as a proof for the tuberculous origin of a questionable lung disease. In the case just reported, acid-fast bacilli were found twice in sputum smears, hence the diagnosis of tuberculosis was made. The necropsy showed no evidence of tuberculosis of the lung. Therefore, the rods found were not tubercle bacilli but saprophytes (Muir and Ritchie [15]) which may be found in exudates from gangrenous processes in lungs. It is, therefore, necessary to judge such positive findings very cautiously, if there is any suspicion of a malignant growth in the lung.

SUMMARY

1. The tumor in this case is a primary gelatinous cylindrical cell carcinoma of the lung, starting from the alveolar epithelium.

2. Two cases reported by Helly and Löhlein as benign adenomas of the lung must be considered as primary gelatinous cylindrical cell carcinomas of the lung starting from the alveolar epithelium.

3. The finding of acid-fast bacilli in the sputum of a patient with gangrenous processes in the lung due to a carcinoma may sometimes lead to the wrong diagnosis of pulmonary tuberculosis.

NOTE. The author wishes to express his appreciation and thanks to Dr. Lloyd Arnold for his help in preparing this paper.

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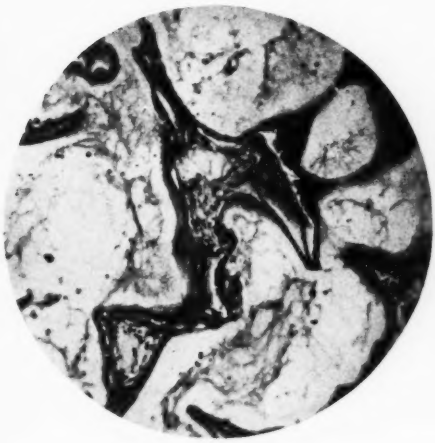
DESCRIPTION OF PLATE

PLATE 20

FIG. 1. Cysts in the thickened pleura showing high cylindrical cells and stringy coagulated content with desquamated tumor cells.

FIG. 2. Lung. Papillary formation, with rosette-like groups of tumor cells in the alveolar spaces; and alveolar septa lined with high cylindrical cells.

FIG. 3. Bronchiole with normal cylindrical epithelium, round cell infiltrations in the interlobular septum, coal-dust pigment and emphysematous lung structure with well preserved alveolor septa which are lined partly with high cylindrical cells.



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Hueper Carcinoma of the Lung

A SUPRAVITAL STUDY OF LEUCOCYTES IN ALLERGIC STATES
A COMPARISON OF DELAYED AND IMMEDIATE INTRA-PLEURAL ANAPHYLACTIC REACTIONS *

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It has long been appreciated that the delayed skin reaction to proteins in rabbits (Arthus phenomenon) possesses some features in common with the tuberculin reaction in guinea-pigs in that both are delayed in onset and are characterized by similar macroscopic lesions, edema, erythema, induration and often necrosis. The authors, with Bradley,[1] have recently studied by supravital staining methods the behavior of reacting leucocytes during intra-pleural tuberculin reactions in guinea-pigs. It has seemed desirable to apply these same methods to the study of delayed and immediate anaphylactic skin reactions, the first being the type of allergy seen in rabbits and the second being the variety found in the guinea-pig.

Consequently a series of rabbits were sensitized by three intraperitoneal injections of horse serum at four day intervals, a total of 10 cc. of antigen. Twenty-one days after the initial injection, all of the rabbits gave strong precipitin reactions up to 1:10,000 dilution of antigen. Pleural exudates were induced in six animals, three sensitized and three controls, by intrapleural injections of 5 cc. of sterile hormone broth to which was added 0.3 cc. of horse serum. The cells of the resultant exudates were examined twenty-four hours later by supravital neutral red and Janus green staining in a constant temperature box. Cell counts are indicated in the following protocols.

RABBIT 774 (sensitized):

living polymorphonuclear neutrophiles, 41 per cent; dead, 44 per cent;
living lymphocytes, none; dead lymphocytes, 2 per cent;
living monocytes, none; dead, 8 per cent;
living clasmatocytes, none; dead, 5 per cent.

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RABBIT 1 (*control*):

living polymorphonuclear neutrophiles, 88 per cent; dead, none;
living polymorphonuclear basophiles, 1 per cent; dead, none;
living monocytes, 4 per cent; dead, none;
living clasmatocytes, 7 per cent; dead, none.

RABBIT 775 (sensitized):

living polymorphonuclear neutrophiles, 2 per cent; dead, 75 per cent;
living polymorphonuclear eosinophiles, none; dead, 6 per cent;
living lymphocytes, none; dead, 1 per cent;
living monocytes, none; dead, 13 per cent;
living clasmatocytes, none; dead, 3 per cent.

RABBIT 2 (*control*):

living polymorphonuclear neutrophiles, 87 per cent; dead, none;
living monocytes, 12 per cent; dead, none;
living clasmatocytes, 1 per cent; dead, none.

RABBIT 776 (sensitized):

living polymorphonuclear neutrophiles, none; dead, 90 per cent;
living lymphocytes, none; dead, 2 per cent;
living monocytes, none; dead, 8 per cent.

RABBIT 3 (*control*):

living polymorphonuclear neutrophiles, 90 per cent; dead, none;
living small lymphocytes, 1 per cent; dead, none;
living monocytes, 9 per cent; dead, none.

Examination of the peripheral blood of these rabbits revealed no dead cells.

Three guinea-pigs were sensitized to horse serum by doses similar to those used for the rabbits. Eighteen days after the last injection attempts were made to induce exudates in these and in three control guinea-pigs by intrapleural injection of 3 or 4 cc. of hormone broth. The following day all animals received additional intrapleural injections of 0.3 cc. horse serum to note its immediate effect on the cells of the preëxisting exudates. The results were not satisfactory. Two of the three sensitized guinea-pigs failed to show fluid; the third one gave a doubtful result, since many leucocytes in the exudates of both the sensitized and the corresponding control guinea-pig were injured, probably from over concentration of the dye but possibly from some normal toxic effect of the horse serum on guinea-pig cells. Two other control guinea-pigs, however, showed no dead cells in their exudates. To avoid any non-specific toxic action of horse serum, a second series of five guinea-pigs were sensitized by

two intraperitoneal injections of chemically pure crystalline egg albumen dialyzed free from ammonium sulphate. The total dose of antigen was 6 cc. of an approximately 10 per cent solution. Eighteen days after the last injection, pleural exudates were produced in the usual manner in four of the animals and twenty-four hours later 0.8 cc. of egg albumen solution was added to the preformed exudate. Two control animals were similarly treated. Cell examinations were made both before and after intrapleural injection of antigen. No dead cells were found before administering the antigen. The cell counts after antigen injection appear in the following protocols.

GUINEA-PIG 431 (sensitized):
living polymorphonuclear neutrophiles, 46 per cent; dead, none;
living polymorphonuclear eosinophiles, 10 per cent; dead, none;
living lymphocytes, 2 per cent; dead, none;
living monocytes, 42 per cent; dead, none.

GUINEA-PIG 1 (*control*):
living polymorphonuclear neutrophiles, 57 per cent; dead, none;
living polymorphonuclear eosinophiles, 4 per cent; dead, none;
living lymphocytes, 2 per cent; dead, none;
living monocytes, 34 per cent; dead, none;
living clasmatocytes, 3 per cent; dead, none.

GUINEA-PIG 432 (sensitized):
living polymorphonuclear neutrophiles, 66 per cent; dead, none;
living polymorphonuclear eosinophiles, 2 per cent; dead, none;
living monocytes, 28 per cent; dead, none;
living clasmatocytes, 4 per cent; dead, none.

GUINEA-PIG 2 (*control*):
living polymorphonuclear neutrophiles, 66 per cent; dead, none;
living polymorphonuclear eosinophiles, 1 per cent; dead, none;
living monocytes, 28 per cent; dead, none;
living clasmatocytes, 5 per cent; dead, none.

GUINEA-PIG 433 (sensitized):
living polymorphonuclear neutrophiles, 64 per cent; dead, 3 per cent;
living polymorphonuclear eosinophiles, 4 per cent; dead, none;
living lymphocytes, 2 per cent; dead, none;
living monocytes, 17 per cent; dead, none;
living clasmatocytes, 10 per cent; dead, none.

GUINEA-PIG 3 (*control*):
living polymorphonuclear neutrophiles, 67 per cent; dead, none;
living polymorphonuclear eosinophiles, 2 per cent; dead, none;
living monocytes, 30 per cent; dead, none;
living clasmatocytes, 1 per cent; dead, none.

RABBIT 1 (*control*):

living polymorphonuclear neutrophiles, 88 per cent; dead, none;
living polymorphonuclear basophiles, 1 per cent; dead, none;
living monocytes, 4 per cent; dead, none;
living clasmatocytes, 7 per cent; dead, none.

RABBIT 775 (sensitized):

living polymorphonuclear neutrophiles, 2 per cent; dead, 75 per cent;
living polymorphonuclear eosinophiles, none; dead, 6 per cent;
living lymphocytes, none; dead, 1 per cent;
living monocytes, none; dead, 13 per cent;
living clasmatocytes, none; dead, 3 per cent.

RABBIT 2 (*control*):

living polymorphonuclear neutrophiles, 87 per cent; dead, none;
living monocytes, 12 per cent; dead, none;
living clasmatocytes, 1 per cent; dead, none.

RABBIT 776 (sensitized):

living polymorphonuclear neutrophiles, none; dead, 90 per cent;
living lymphocytes, none; dead, 2 per cent;
living monocytes, none; dead, 8 per cent.

RABBIT 3 (*control*):

living polymorphonuclear neutrophiles, 90 per cent; dead, none;
living small lymphocytes, 1 per cent; dead, none;
living monocytes, 9 per cent; dead, none.

Examination of the peripheral blood of these rabbits revealed no dead cells.

Three guinea-pigs were sensitized to horse serum by doses similar to those used for the rabbits. Eighteen days after the last injection attempts were made to induce exudates in these and in three control guinea-pigs by intrapleural injection of 3 or 4 cc. of hormone broth. The following day all animals received additional intrapleural injections of 0.3 cc. horse serum to note its immediate effect on the cells of the preëxisting exudates. The results were not satisfactory. Two of the three sensitized guinea-pigs failed to show fluid; the third one gave a doubtful result, since many leucocytes in the exudates of both the sensitized and the corresponding control guinea-pig were injured, probably from over concentration of the dye but possibly from some normal toxic effect of the horse serum on guinea-pig cells. Two other control guinea-pigs, however, showed no dead cells in their exudates. To avoid any non-specific toxic action of horse serum, a second series of five guinea-pigs were sensitized by

two intraperitoneal injections of chemically pure crystalline egg albumen dialyzed free from ammonium sulphate. The total dose of antigen was 6 cc. of an approximately 10 per cent solution. Eighteen days after the last injection, pleural exudates were produced in the usual manner in four of the animals and twenty-four hours later 0.8 cc. of egg albumen solution was added to the preformed exudate. Two control animals were similarly treated. Cell examinations were made both before and after intrapleural injection of antigen. No dead cells were found before administering the antigen. The cell counts after antigen injection appear in the following protocols.

GUINEA-PIG 431 (sensitized):
living polymorphonuclear neutrophiles, 46 per cent; dead, none;
living polymorphonuclear eosinophiles, 10 per cent; dead, none;
living lymphocytes, 2 per cent; dead, none;
living monocytes, 42 per cent; dead, none.

GUINEA-PIG 1 (*control*):
living polymorphonuclear neutrophiles, 57 per cent; dead, none;
living polymorphonuclear eosinophiles, 4 per cent; dead, none;
living lymphocytes, 2 per cent; dead, none;
living monocytes, 34 per cent; dead, none;
living clasmatocytes, 3 per cent; dead, none.

GUINEA-PIG 432 (sensitized):
living polymorphonuclear neutrophiles, 66 per cent; dead, none;
living polymorphonuclear eosinophiles, 2 per cent; dead, none;
living monocytes, 28 per cent; dead, none;
living clasmatocytes, 4 per cent; dead, none.

GUINEA-PIG 2 (*control*):
living polymorphonuclear neutrophiles, 66 per cent; dead, none;
living polymorphonuclear eosinophiles, 1 per cent; dead, none;
living monocytes, 28 per cent; dead, none;
living clasmatocytes, 5 per cent; dead, none.

GUINEA-PIG 433 (sensitized):
living polymorphonuclear neutrophiles, 64 per cent; dead, 3 per cent;
living polymorphonuclear eosinophiles, 4 per cent; dead, none;
living lymphocytes, 2 per cent; dead, none;
living monocytes, 17 per cent; dead, none;
living clasmatocytes, 10 per cent; dead, none.

GUINEA-PIG 3 (*control*):
living polymorphonuclear neutrophiles, 67 per cent; dead, none;
living polymorphonuclear eosinophiles, 2 per cent; dead, none;
living monocytes, 30 per cent; dead, none;
living clasmatocytes, 1 per cent; dead, none.

In order to verify the sensitization, guinea-pig 432 was given 1 cc. of antigen intravenously after the pleural tap had been made; the animal died a typical anaphylactic death in about five minutes. Guinea-pig 433 died an anaphylactic death about forty-five minutes after receiving the antigen intrapleurally; necropsy showed distended, rigid lungs, a heart still beating and small gastric hemorrhages; microscopically lungs were markedly edematous. The cells of the exudates of both of these animals were alive and motile three hours after the death of the guinea-pig and of course were all that time in contact with antigen.

One guinea-pig received 0.3 cc. of antigen in 4 cc. of hormone broth in the right pleural cavity in order that any delayed reaction might be studied. In twenty-four hours the fluid contained only living cells. The cell count was as follows:

living polymorphonuclear neutrophiles, 77 per cent; dead, none;
living polymorphonuclear eosinophiles, 1 per cent; dead, none;
living small lymphocytes, 1 per cent; dead, none;
living monocytes, 16 per cent; dead, none;
living clasmatocytes, 5 per cent; dead, none.

The question was then raised as to whether the rabbits sensitized to horse serum would show any immediate reaction. Consequently, using the same rabbits employed for the study of the delayed reaction, the precipitin titer was brought back to a high level (1:10,000) by two additional injections of horse serum intraperitoneally. Pleural fluids were produced with hormone broth and in twenty-four hours 1 cc. of horse serum was added to the exudate. The rabbits were tapped twenty to thirty minutes later. One animal gave no fluid; the other two yielded an exudate containing only living cells.

RABBIT 774 (sensitized):

living polymorphonuclear neutrophiles, 42 per cent; dead, none;
living polymorphonuclear basophiles, 1 per cent; dead, none;
living polymorphonuclear eosinophiles, 1 per cent; dead, none;
living lymphoocytes, 1 per cent; dead, none;
living monocytes, 23 per cent; dead, none;
living clasmatocytes, 32 per cent, dead, none.

RABBIT 775 (sensitized):

living polymorphonuclear neutrophiles, 50 per cent; dead, none;
living lymphocytes, 6 per cent; dead, none;
living monocytes, 15 per cent; dead, none;
living clasmatocytes, 26 per cent; dead, none;
living serosal cells, 3 per cent; dead, none.

Summarizing the above results, it will be seen that in rabbits the leucocytes are killed after contact with the antigen for twenty-four hours but not after a shorter period, *i.e.*, thirty minutes. In guinea-pigs, on the other hand, the leucocytes are not injured at either period. The differences in the results in these two animals are interesting and significant in view of the difference in their skin reactions. As is well known, a sensitized guinea-pig gives an immediate skin reaction on reinjection of the antigen intradermally; this skin reaction is transient and causes no lasting injury such as necrosis. The guinea-pig under ordinary conditions, at least, does not give a delayed skin reaction. A sensitized rabbit, on the other hand, never gives an immediate skin reaction but does give a delayed skin reaction which is more than transient and in a highly sensitized animal leads to definite injury such as necrosis. The cause for the variation in the two animals is not yet clear; it has been suggested by some that the difference is dependent on the inequality in amount of circulating antibodies, for in guinea-pigs the titer of these is usually low in comparison to the titer in rabbits. However, whatever the explanation of this difference in type of reaction may be, in the above experiments an analogous condition as regards their leucocytes apparently holds true, *i.e.*, the leucocytes of the guinea-pig are uninjured by antigen whereas those of the rabbit are killed. This apparently is a fundamental difference and may throw some light on the dissimilarity in the types of skin reactions, for it seems improbable that these cells alone, the leucocytes, should be the only cells that become sensitive to the antigen. Here again the presence of a large amount of circulating antibody can conceivably be the cause of this difference; further work along this line is in progress.

On comparing these results with those obtained by the authors in working with tuberculin sensitization in guinea-pigs, a striking similarity will be noted. In a tuberculin reactive guinea-pig the leucocytes are killed on contact with tuberculin for twenty-four hours. The skin tuberculin reaction in a guinea-pig is of course a delayed reaction and the analogy with a rabbit sensitive to horse serum, giving a delayed reaction, whose leucocytes are killed by the antigen, is evident.

If we consider the death of leucocytes on exposure to antigen as due to some definite inherent cause dependent on factors other than circulating antibody content, then we are faced with the following

situation. In protein sensitized guinea-pigs that give only an immediate reaction, the leucocytes are uninjured by antigen. In protein sensitized rabbits that give only a delayed reaction, the leucocytes are killed by the antigen. In guinea-pigs reactive to tuberculin, that give only a delayed reaction, the leucocytes are killed by the antigen. How leucocytes of tuberculin sensitive rabbits behave in contact with tuberculin is a question which will be dealt with in the near future.

Two small histologic details, although wholly apart from the general subject of this communication, nevertheless deserve reporting. The first of these concerns the question of phagocytized polymorphonuclears. The writers had always assumed from study of fixed preparations that when once a polymorphonuclear had been taken up by a monocyte or clasmatocyte, the phagocytized cell died. Probably in most instances this is true although death is not immediate and many living polymorphonuclears are engulfed to be destroyed later. On one occasion during the present study, a phagocytized polymorphonuclear neutrophile was observed to free itself completely from a clasmatocyte and to escape. The other point of note may be described briefly as follows.

While studying the pleural effusions of rabbits for evidence of an immediate type of reaction, we observed what appeared to be direct cell division in two polymorphonuclear leucocytes; one of these divisions was seen too late to follow the method, but the other was observed in fair detail: a polymorphonuclear neutrophile with a triple nuclear mass was seen to segment partially, one of the nuclear masses passing through the constricted zone and two remaining behind; granules could be observed passing through the constricted portion which gradually became greatly attenuated and finally ruptured, the cells moving off in opposite directions. No evidences of mitotic phenomena were seen. So far as we are aware, neither of the above observations has hitherto been recorded although no survey of the literature has been made.

Summary and Conclusions

In the delayed intrapleural anaphylactic reaction in rabbits sensitive to horse serum, reacting leucocytes are largely killed.

In this respect, the reaction parallels the intrapleural tuberculin reaction in guinea-pigs.

Attempts to induce an immediate intrapleural anaphylactic reaction in rabbits resulted in no apparent cell injury.

In sensitized guinea-pigs no cell injury either immediate or delayed was observed when antigen was introduced into the pleural cavity.

Evidence gleaned from this study accords well with facts already known about delayed and immediate anaphylaxis.

REFERENCE

1. Stewart, F. W., Long, Perrin H., and Bradley, John I. The fate of reacting leucocytes in the tuberculin and reinfection reactions. *Am. J. Path.* (1926), ii, 47.